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The Cellular and Molecular Basis of Direction Selectivity of Aδ-LTMRs

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Summary

The perception of touch, including the direction of stimulus movement across the skin, begins with activation of low-threshold mechanosensory neurons (LTMRs) that innervate the skin. Here, we show that murine Aδ-LTMRs are preferentially tuned to deflection of body hairs in the caudal-to-rostral direction. This tuning property is explained by the finding that Aδ-LTMR lanceolate endings around hair follicles are polarized; they are concentrated on the caudal (downward) side of each hair follicle. The neurotrophic factor BDNF is synthesized in epithelial cells on the caudal, but not rostral, side of hair follicles, in close proximity to Aδ-LTMR lanceolate endings, which express TrkB. Moreover, ablation of BDNF in hair follicle epithelial cells disrupts polarization of Aδ-LTMR lanceolate endings and results in randomization of Aδ-LTMR responses to hair deflection. Thus, BDNF-TrkB signaling directs polarization of Aδ-LTMR lanceolate endings, which underlies direction-selective responsiveness of Aδ-LTMRs to hair deflection.

Introduction

Our ability to detect the direction of movement of stimuli in our sensory world is critical to survival; therefore, it is no surprise that a large portion of our sensory systems is devoted to

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the perception of stimulus movement across our environmental landscape. In the visual system, direction-selective retinal ganglion cells (DS-RGCs) and higher order visual centers, such as the visual area middle temporal (MT), are concerned with image movement across visual space (Wei and Feller, 2011). In the auditory system, the principal nuclei of the superior olivary complex process interaural time differences, which are critical for sound localization (Grothe et al., 2010). While the cells and circuits underlying detection and processing of visual and auditory direction-selective stimuli are becoming understood, little is known about how the direction of movement of stimuli acting on the skin, which is our largest sensory organ, is detected and processed.

The sense of touch allows us to recognize and manipulate objects held in our hands, detect innocuous or potentially harmful stimuli acting upon our bodies, and it enables physical communication for social bonding, sexual pleasure, and procreation. The neurobiological steps leading to the perception of touch begin with activation of low-threshold mechanoreceptors (LTMRs) by physical stimuli acting on the skin. LTMR cell bodies reside within dorsal root ganglia (DRG) and trigeminal ganglia and have one axonal branch that extends to the periphery and associates with a cutaneous mechanosensory end organ and another branch that penetrates the spinal cord and forms synapses upon second order neurons in the spinal cord dorsal horn and, in some cases, the dorsal column nuclei of the brainstem. LTMRs are sensitive to innocuous indentation, stroking, vibration, or stretch of the skin, and the deflection of hair follicles. Current challenges include defining mechanisms of unique tuning properties and functions of LTMR subtypes and determining how ensembles of LTMR activities are represented, integrated and processed in the CNS to give rise to the perception of touch.

Adrian and Zotterman (1926) first described the electrophysiological properties of sensory neurons that respond to hairy skin stimulation and their work laid the foundation and subsequent classification of the main LTMR types that associate with mammalian hairy skin (Zotterman, 1939). Aβ RA-LTMRs, field receptors (F-LTMRs), Aβ SAI-LTMRs, down (D-) hair follicle afferents/Aδ-LTMRs, and C-LTMRs were initially defined based on stimulus response characteristics, the conduction velocity of their action potentials, adaptation properties, and the morphology of hairs with which they associate (Brown and Iggo, 1967; Burgess et al., 1968; Zotterman, 1939). Aβ RA-LTMRs and Aβ SAI-LTMRs have large myelinated axons, fast conduction velocities, and adapt rapidly or slowly, respectively, during sustained mechanical stimulation of the skin. While Aβ RA-LTMR subtypes are velocity detectors that respond to skin indentation, movement of stimuli across the skin, and defection of hair follicles, Aβ SAI-LTMRs terminate in Merkel discs of touch domes, respond preferentially to skin indentation, and report on the static nature of tactile stimulations (Koltzenburg et al., 1997; Woodbury and Koerber, 2007). Although the morphology, physiology, and function of F-LTMRs are less well understood and they are not yet genetically identified, in cats they display Aβ conduction velocities, exhibit large receptive fields, and while they are highly sensitive to stroking of hairy skin, they respond poorly to skin indentation and deflection of individual hairs. A fourth hairy skin LTMR type, Aδ-LTMRs, are the most sensitive of the LTMRs, have lightly myelinated axons with an intermediate conduction velocity and, like Aβ RA-LTMRs, they are velocity detectors that rapidly adapt to sustained stimulation. Originally thought to associate exclusively with

small hair types, termed down hairs in cats, it is now established that Aδ-LTMR responses are elicited following movement of multiple hair types (Brown and Iggo, 1967; Burgess et al., 1968; Horch et al., 1977). Finally, C-LTMRs exhibit a slow conduction velocity and an intermediate rate of adaptation, and recent work in humans suggests an involvement in pleasurable or emotional touch because they are optimally tuned to stroking of the skin at rates that are deemed pleasurable (Hamann, 1995; Horch et al., 1977; Olausson et al., 2002). While each of the main hairy skin LTMRs is sensitive to innocuous touch of the skin or body hairs, the mechanisms by which LTMR subtypes are differentially tuned to specific-touch stimuli remains incompletely understood. Thus, we previously employed a molecular genetic labeling strategy to study peripheral and central axonal projections of distinct LTMR subtypes with the goal of uncovering morphological correlates of LTMR subtype response properties and the functional organization of LTMR projections (Li et al., 2011). The peripheral terminals of Aβ RA-LTMRs, Aδ-LTMRS, and C-LTMRs in murine back hairy skin form lanceolate axonal endings in unique combinations with the three main types of hair follicles of the mouse; guard, awl/auchene, and zigzag hairs. Guard hair follicles, the largest and least abundant, comprising ∼1% of all murine hairs, receive rich innervation by Aβ RA-LTMR lanceolate endings and also associate with Aβ SAI-LTMR endings, which terminate upon Merkel discs in touch dome complexes. Awl/auchene hairs make up ∼20% of hair follicles and are triply innervated by interdigitated Aβ RA-LTMR, Aδ-LTMR, and C-LTMR lanceolate endings. The most abundant and smallest hairs, zigzag hairs, comprise ∼80% of hair follicles and are innervated by interdigitated Aδ-LTMR and C-LTMR lanceolate endings. Each of the three hair follicle types is also surrounded by circumferential endings belonging to neurons of unknown physiological properties. Thus, each of the three distinct hair types is associated with unique combinations of LTMR axonal endings, thereby rendering them neurophysiologically distinct (Li et al., 2011).

The contributions of LTMR subtypes and downstream spinal cord and brainstem circuit components to the capture, processing, and perception of the direction of stimulus movement across the skin are not known. LTMRs that innervate hairy skin are themselves candidates for having direction-selective tuning properties because each hairy skin LTMR subtype is associated with one or more hair follicle types and several are highly sensitive to hairy skin stroking and hair deflection. Moreover, cat, rat, and mouse trigeminal mechanosensory neurons that associate with whisker follicles of mystacial pads exhibit direction-selective responses to whisker deflection (Gottschaldt and Vahle-Hinz, 1981; Kwegyir-Afful et al., 2008; Lichtenstein et al., 1990). On the other hand, attempts to address the issue of direction selectivity of hairy skin LTMRs of cats, rabbits, and primates have suggested that LTMRs are (Brown and Iggo, 1967; Maruhashi et al., 1952; Tuckett, 1978) or are not (Essick and Whitsel, 1985; Greenspan, 1992; Hyvärinen and Poranen, 1978; Whitsel et al., 1972) sensitive to the direction of deflection of normal hair follicles. One study that asked whether feline hairy skin Aδ-LTMRs exhibit direction-selective responses concluded that these neurons may be direction-selective but that it is difficult to ascertain because of their ultrasensitive response property (Ray et al., 1985). Indeed, no one LTMR subtype has been unequivocally shown to be differentially sensitive to the direction of movement of objects across hairy skin, leading to the idea that information about direction of stimulus movement is represented exclusively by the firing patterns evoked in populations of

mechanoreceptors activated by a moving tactile stimulus, rather than by direction selectivity of individual LTMR subtypes (Essick and Edin, 1995). Thus, whether Aδ-LTMRs, the most sensitive of hairy skin mechanoreceptors, or other hairy skin LTMR subtypes exhibit direction-selective responses to hair deflection or skin stroking remains an open question, and quantitative measures of LTMR response properties in conjunction with sensitive hair deflection paradigms are needed to address this question.

Here, using novel methods for deflecting individual hair follicles in the four cardinal directions relative to the body axis of the mouse, we report that Aδ-LTMRs are more sensitive to hair deflection in the caudal-to-rostral (R) direction than in the rostral-to-caudal (C) direction. A morphological correlate of this unique tuning property is a striking enrichment of Aδ-LTMR lanceolate endings on the caudal side of awl/auchene and zigzag hair follicles. Interestingly, expression of the neurotrophic growth factor BDNF, which is well known to control axonal growth and target innervation, is localized to caudal side epithelial cells of these hair follicles, the BDNF receptor TrkB is highly expressed in Aδ-LTMRs, and conditional ablation of *BDNF* in skin epithelial cells leads to a loss of Aδ-LTMR lanceolate ending polarization. As a result of loss of lanceolate ending polarization, direction-selective tuning of Aδ-LTMRs to hair deflection is randomized in these conditional BDNF mutant mice. Thus, Aδ-LTMRs are direction-selective hairy skin mechanoreceptors, and BDNF-to-TrkB signaling between hair follicle epithelial cells and Aδ-LTMRs lanceolate endings establishes Aδ-LTMR terminal polarization and their direction selectivity to hair deflection.

Results

Aδ-LTMRs Are Tuned to the Direction of Hair Deflection

We previously established molecular-genetic tools to investigate Aβ RA-, Aδ-, and C-LTMRs and demonstrated that these sensory neuron subtypes form interdigitated longitudinal lanceolate endings in close association with select types of hair follicles in mouse hairy skin. Utilizing a $TrkB^{GFP}$ knockin mouse line, $TrkB^{+}$ DRG neurons of adult mice were found to be Aδ-LTMRs, which comprise ∼7% of adult thoracic DRG neurons and exhibit central axonal projections that terminate within lamina IIiv/III of the spinal cord dorsal horn. The peripheral projections of Aδ-LTMRs form longitudinal lanceolate endings associated with both awl/auchene and zigzag hairs of trunk hairy skin (Li et al., 2011). To facilitate examination of the physiological and morphological properties of individually labeled Aδ-LTMRs, we generated a $TrkB^{CreER}$ knockin mouse line in which a fusion cassette consisting of Cre recombinase and a triple mutant form of the human estrogen receptor (CreERT2) was introduced via homologous recombination into the first coding exon of the *TrkB* gene (Figure S1 available online). Treatment of $TrkB^{CreER}$; $Rosa26^{LSL\text{-}tdTomato}$ or $TrkB^{CreER}$;$Rosa26^{iAP}$ mice (Badea et al., 2009; Madisen et al., 2010) with a single injection of 4-hydroxy-tamoxifen (4-HT) at embryonic day 10 (E10) or E13 led to constitutive expression of tdTomato or alkaline phosphatase (AP), respectively, in medium diameter DRG neurons of adult mice, which express TrkB (n = 194 cells labeled in $TrkB^{CreER}$;$Rosa26^{LSL\text{-}tdTomato}$ mice; Figure 1A). As expected, the peripheral axons of sparsely labeled neurons form longitudinal lanceolate endings at awl/auchene and zigzag

hairs. Their central projections terminate in bouton-rich “flame-shaped arbors” that are continuous throughout the rostral-caudal axis of laminae IIiv/III of the spinal cord dorsal horn (Figure S2E). The longitudinal extent of Aδ-LTMR central terminations range from 250 μm in cervical and lumbar regions to 500 μm in thoracic segments (Figures S2A–S2D). In addition, sharp electrode recordings of $TrkB^{CreER}$-labeled neurons in intact ex vivo and in vivo preparations revealed that they exhibit Aδ-LTMR physiological properties, with narrow uninflected somal spikes, Aδ conduction velocities, low mechanical thresholds, and rapidly adapting responses to indentation of the skin (Figure S2F). Thus, $TrkB^{CreER}$ mice specifically label Aδ-LTMRs enabling versatile genetic access to this neuronal population.

Because Aδ-LTMRs terminate in lanceolate endings associated with hair follicles, we next sought to define their response properties with respect to hair follicle deflection. As shown previously in cats, rabbits, rats, and mice, Aδ-LTMRs are exquisitely sensitive and exhibit robust, rapidly adapting responses to maintained stimuli. These neurons respond throughout the dynamic phase of stimuli, providing bursts of spikes at both the onset and termination of sustained mechanical stimuli. To investigate Aδ-LTMR responses to hair deflection, we used fine probes to deflect small clusters of 3-6 hairs within the receptive fields of $TrkB^{GFP}$-labeled Aδ-LTMRs. As observed with sustained indentation, Aδ-LTMRs responded briskly throughout the movement of hairs but adapted rapidly when movement ceased (Figure 1B). Remarkably, cells were found to respond more strongly to deflection of groups of hairs in the caudal-to-rostral (R) direction, compared to the rostral-to-caudal (C) direction.

Because deflection of hair clusters subjected individual hairs within the clusters to unpredictable movements, we next asked whether direction-selective responses of Aδ-LTMRs are observed following controlled deflection of individual hairs. High-resolution mapping was performed throughout the cell's receptive field, using controlled deflections in the four cardinal directions, as clusters were successively refined down to a single hair. These fine-grained analyses, akin to fiber-teasing techniques in extracellular recordings from nerve bundles, revealed that the large receptive fields of Aδ-LTMRs represent a mosaic patchwork; movements of doublets and triplets elicited responses that were often indistinguishable from single hairs; conversely, large numbers of individual hairs within the boundaries of receptive fields often elicited no response in the cell (data not shown) despite being adjacent to and/or surrounded by hairs that did, suggesting remarkably little sensory coupling between adjacent hair follicles in the dermis.

As observed for Aδ-LTMRs responses to deflection of groups of hairs, a comparison of Aδ-LTMR responses to deflection of individual hairs revealed a pronounced direction-selective tuning property. Optimal Aδ-LTMR responses were generally elicited by movement in the R direction; for the majority of single hairs and doublets tested, R tuning was extremely sharp, and responses to movement in the C direction were frequently nonexistent. Deflections in the orthogonal plane revealed that many hairs also elicited good responses following deflection in the ventral-to-dorsal (D) direction or alternatively, the dorsal-to-ventral (V) direction, in addition to the R direction, suggesting that our manipulator may not have been aligned with the optimal vector for that hair (Figures 1C and 1D). Combining the responses from all spots tested throughout receptive fields of Aδ-LTMRs, from single hairs to groups of five, revealed strong directional tuning overall; the average number of spikes elicited in

Aδ-LTMRs by R deflections of hairs was over 3-fold more than the number elicited from C deflections of the same magnitude ($p < 0.01$, Figure 1E). Finally, we performed similar analyses using an in vivo preparation that enabled recordings of trigeminal ganglion $TrkB^{CreER}$-labeled Aδ-LTMRs, which innervate hairy skin of the head. Although facial hairs exhibited greater heterogeneity in orientation than trunk hairs, trigeminal Aδ-LTMRs also exhibit direction-selective patterns of activation following hair deflection (Figures 1F and 1G). Direction selectivity in response to hair deflection is not a property of all LTMR subtypes because in vivo recordings of trigeminal Aβ RA-LTMRs, which form lanceolate endings associated with guard and awl/auchene hair follicles, failed to show a directional preference (Figure S3). Thus, DRG and trigeminal ganglion Aδ-LTMRs are ultrasensitive mechanosensory neurons optimally tuned to hair movement in the R direction (Figure 1H).

Aδ-LTMRs Endings at Hair Follicles Are Polarized

In the visual system, a small subset of direction-selective retinal ganglion cells (DS-RGCs) exhibit polarized dendrites that are oriented in the direction of their optimal responses, and this morphological feature may underlie direction-selective capture of visual images moving across the receptive fields of this subset (Kim et al., 2008; Trenholm et al., 2011). To begin to ask whether there is a morphological basis of Aδ-LTMR direction-selective tuning to hair deflection, the peripheral axonal ending morphology of individual Aδ-LTMRs was visualized. To accomplish this, we used *TrkB*^{CreER};*Rosa26*^{iAP} and *TrkB*^{CreER};*Rosa26*$^{LSL\text{-}tdTomato}$ mice and induced expression of AP or tdTomato, respectively, in small numbers of Aδ-LTMRs thereby enabling detailed examination of their cutaneous axonal morphologies. Sections of back hairy skin collected from *TrkB*^{CreER};*Rosa26*^{iAP} and *TrkB*^{CreER};*Rosa26*$^{LSL\text{-}tdTomato}$ mice were analyzed using either whole mount AP or immunohistochemical staining techniques. The peripheral axonal branches of individual adult Aδ-LTMRs were found to arborize and form longitudinal lanceolate endings associated an average of 35 zigzag and awl/auchene hair follicles, encompassing a skin area of 0.6-0.8 mm^2 ($n = 3$; Figure 2A). Interestingly, both low and high magnification images of Aδ-LTMR projections in the skin revealed a marked polarization of their lanceolate endings surrounding hair follicles. These lanceolate endings are greatly enriched on the caudal side of hair follicles (Figure 2B). Lanceolate ending polarization is unique to Aδ-LTMRs as neither C-LTMRs nor Aβ RA-LTMRs exhibit appreciable polarization (Figures 2C and 2D). Vector analysis reveals a highly significant polarization of Aδ-LTMR endings on the caudal sides of awl/auchene and zigzag follicles in back hairy skin (Figure 2E). This polarization may provide a structural basis of selective responses of Aδ-LTMRs to hair deflection in the R direction.

BDNF Expression Is Polarized and Concentrated on the Caudal Side of Hair Follicles in Close Association with Aδ-LTMR Lanceolate Endings

We next sought to identify asymmetrically localized cue(s) that instruct the polarization of Aδ-LTMR lanceolate processes around hair follicles because ablation of such a cue, as a means to disrupt lanceolate ending polarization, would allow us to ask whether lanceolate ending polarization underlies Aδ-LTMR direction-selective responses. Our analysis focused on the temporal and spatial patterns of expression of the neurotrophins in hairy skin during Aδ-LTMR development because robust expression of the neurotrophin receptor TrkB is a

distinguishing feature of Aδ-LTMRs and because neurotrophins are well known to control sensory axon development and target field innervation. TrkB has two main ligands, brain-derived neurotrophic factor (BDNF) and neurotrophin-4 (NT4), while a third neurotrophin, neurotrophin-3 (NT3), can bind TrkB with low affinity (Klein et al., 1991, 1992). To examine the expression patterns of BDNF, NT4, and NT3 in the context of hair follicle development and sensory neuron innervation, we used $BDNF^{LacZ}$, $NT3^{LacZ}$, and $NT4^{LacZ}$ knockin mouse lines (Fariñas et al., 1994; Gorski et al., 2003; Liu et al., 2012). Whole-mount X-gal staining of $BDNF^{LacZ}$ and $NT3^{LacZ}$ embryos revealed robust LacZ expression associated with developing hair follicles in animals of both genotypes, whereas $NT4^{LacZ}$ mice failed to exhibit appreciable expression. LacZ expression in both $BDNF^{LacZ}$ and $NT3^{LacZ}$ embryos, but not $NT4^{LacZ}$ embryos, was detected during the first wave of hair follicle morphogenesis (∼E14) and persisted through the second (E15) and third waves (E18) (Figures 3A, 3B, S4A, and S4B). Hair follicle-associated BDNF and NT3 expression was also observed in adulthood (data not shown). Strikingly, asymmetrically localized BDNF concentrated on the caudal side of hair follicles was observed just below the sebaceous gland in a bulbous region of the follicle, in close proximately to Aδ-LTMR lanceolate endings (Figure 3C). To determine whether BDNF is produced in hair follicle cells of epithelial origin, mice in which Cre recombinase is expressed in skin keratinocytes ($K5^{Cre}$) were crossed with mice harboring a $BDNF^{flox}$ conditional reporter allele (Gorski et al., 2003; Ramirez et al., 2004). Thus, following Cre-mediated excision, one copy of BDNF is excised and expression of a functional LacZ reporter cassette becomes activated. Indeed, robust, asymmetrically localized LacZ staining was observed in $K5^{Cre}$; $BDNF^{flox}$ mice (Figures 3F and S4C), indicating that BDNF is produced in epithelial cells of the caudal region of hair follicles. In contrast, NT3 exhibits a symmetric pattern of expression around zigzag and awl/auchene hair follicles, although it is asymmetrically expressed around guard hairs, which are not innervated by Aδ-LTMRs (Figures 3D and 3E). Thus, while both BDNF and NT3 are expressed in epithelial cells of awl/auchene and zigzag hair follicles, BDNF, but not NT3, is asymmetrically distributed in epithelial cells surrounding these follicles being greatly enriched on the hair follicle caudal side, which harbors Aδ-LTMR endings.

To directly compare the spatial and temporal relationships between BDNF expression and nascent Aδ-LTMR lanceolate endings associated with hair follicles during development, we next generated $BDNF^{LacZ}$; $TrkB^{CreER}$;$Rosa26^{LSL\text{-}tdTomato}$ mice. These mice were given a low-dose of tamoxifen (1 mg) at E13.5 to enable simultaneous visualization of BDNF expression patterns and nascent Aδ-LTMR axonal endings around hair follicles. Aδ-LTMR axons reach the skin prior to birth and associate with hair follicles at neonatal times (data not shown). Subsequently, beginning approximately postnatal day 1 (P1), lanceolate processes emerge from rudimentary processes, circumferentially oriented around hair follicles. By P7, fully formed Aδ-LTMR lanceolate endings concentrated on the caudal sides of hair follicles are observed. Interestingly, during the time of Aδ-LTMR axonal ending growth and maturation, the locations of lanceolate processes and BDNF expression is strikingly coincident. At P5, when nascent lanceolate endings are extending along the longitudinal axis of hair follicles, they are intimately associated with hair follicle epithelial cells that express BDNF and not with epithelial cells that lack BDNF (Figure 4). Thus, hair follicle epithelial cell-derived BDNF is temporally and spatially positioned to serve as a growth and guidance

cue for Aδ-LTMR lanceolate endings during their period of extension and polarization around hair follicles.

BDNF Expression in Hair Follicle Epithelial Cells Is Required for Polarization of Aδ-LTMR Endings

The robust expression of BDNF in caudally located hair follicle epithelial cells that are in close proximity to developing lanceolate endings, and of TrkB in Aδ-LTMRs, suggests a role for BDNF-TrkB signaling in Aδ-LTMR lanceolate ending polarization. Therefore, we next asked whether TrkB and its ligand BDNF control development of Aδ-LTMRs and the extent to which BDNF-TrkB signaling contributes to Aδ-LTMR lanceolate ending polarity. Indeed, through monitoring GFP expression from the $TrkB^{GFP}$ allele as a general readout of the integrity of Aδ-LTMRs, a nearly complete loss of GFP^+ neurons in $TrkB^{GFP}$ homozygous null mutants at the day of birth (P0) was observed. Likewise, BDNF null mutants exhibited a 66% reduction in the number of GFP^+ Aδ-LTMRs (Figures S5A and S5B). Thus, embryonic BDNF-TrkB signaling is necessary for Aδ-LTMR development. We next asked whether BDNF produced in hair follicle epithelial cells contributes to Aδ-LTMR development. The $TrkB^{GFP}$ allele was again used to monitor Aδ-LTMR integrity, and the number of thoracic level GFP^+ DRG neurons in $BDNF^{f/f}$ mice expressing Cre recombinase in each of three general cells types that normally express this ligand were counted. To conditionally ablate BDNF in epithelial cells, $K5^{Cre}$; $BDNF^{flox/flox}$; $TrkB^{GFP}$ were generated. The number of GFP^+ neurons in adult $K5^{Cre}$, $BDNF^{flox/flox}$; $TrkB^{GFP}$ were comparable to their littermate controls (Figures S5C and S5D). In contrast, mesenchyme-derived BDNF is required for Aδ-LTMR development because a 66% reduction of GFP^+ neurons was observed in P0 T^{Cre}; $BDNF^{flox/flox}$; $TrkB^{GFP}$ animals, which express Cre recombinase in cells derived from mesoderm (Perantoni et al., 2005) (Figure S5D). $Wnt1^{Cre}$, $BDNF^{flox/flox}$; $TrkB^{GFP}$, which lack BDNF in all DRG neurons and Schwann cells, exhibited a normal complement of Aδ-LTMRs. These findings indicate that BDNF emanating from the mesenchyme, and not hair follicle epithelial cells or DRG neurons themselves or their associated Schwann cells, is essential for maturation and general development of Aδ-LTMRs.

The normal complement of Aδ-LTMRs found *in* $K5^{Cre}$, $BDNF^{flox/flox}$; $TrkB^{GFP}$ mice indicates that epithelial cell-derived BDNF is dispensable for general maturation and survival of these neurons. Therefore, we next used $K5^{Cre}$, $BDNF^{flox/flox}$; $TrkB^{GFP}$ mice to assess the role of BDNF expressed in hair follicle-associated epithelial cells for hair follicle innervation and polarization of Aδ-LTMR lanceolate endings. While polarization of Aδ-LTMR lanceolate endings was readily apparent in control animals, lanceolate endings of $K5^{Cre}$, $BDNF^{flox/flox}$; $TrkB^{GFP}$ mice, while present, showed a marked loss of polarization (Figures 5A and 5B). Some hair follicles exhibited a bias of endings on one side or the other, but the overall pattern of lanceolate ending organization was randomized with respect to hair follicle orientation (Figure 5C). Thus, while hair follicle epithelial cell-derived BDNF is dispensable for general maturation and survival of Aδ-LTMRs, and for hairy skin innervation, it is essential for morphological polarization of Aδ-LTMR lanceolate endings on the caudal sides of awl/auchene and zigzag hair follicles.

BDNF Expression in Hair Follicle Epithelial Cells Is Required for Direction-Selective Responses of Aδ-LTMRs

The lack of Aδ-LTMR lanceolate ending polarization in $K5^{Cre}$; $BDNF^{flox/flox}$; $TrkB^{GFP}$ mice renders these animals valuable for asking whether morphological polarization of lanceolate endings underlies direction-selective tuning of Aδ-LTMRs to hair deflection. Therefore, we next used $K5^{Cre}$; $BDNF^{flox/flox}$; $TrkB^{GFP}$ mice for ex vivo skin-nerve recordings to address this possibility. Remarkably, in $K5^{Cre}$; $BDNF^{flox/flox}$; $TrkB^{GFP}$ mice, Aδ-LTMR responses to oriented hair deflections were randomized with respect to the direction of hair deflection (Figure 6B). While many individual hairs in the receptive fields of Aδ-LTMRs in $K5^{Cre}$; $BDNF^{flox/flox}$; $TrkB^{GFP}$ mice exhibited preferential tuning to deflection in the R direction, as in neurons from wild-type mice, others showed preference to deflection in the opposite, C direction; still others showed preferential tuning to directions of hair movement that were not seen among hairs innervated by Aδ-LTMRs in wild-type mice (Figure 6C). Quantification of the results from fine-grained analyses throughout the receptive fields of Aδ-LTMRs showed that responses in the mutants were randomized with respect to direction of hair deflection (Figure 6D). On the other hand, the somal spikes, peripheral conduction velocities, adaptation properties to sustained stimuli, sensitivity to cooling, and mechanical thresholds of Aδ-LTMRs to stimulation with von Frey filaments were normal in $K5^{Cre}$; $BDNF^{flox/flox}$; $TrkB^{GFP}$ (Figure S6). Thus, while Aδ-LTMR sensitivity and basic physiological properties are intact in the absence of hair follicle epithelial cell-derived BDNF, they fail to exhibit direction-selective responses to hair follicle deflection. We conclude that the morphological polarization Aδ-LTMR lanceolate endings associated with hair follicles underlies direction-selective tuning of Aδ-LTMRs to hair deflection.

Discussion

Direction-selective responses to sensory stimuli is a hallmark feature of several sensory systems, and elucidating the mechanisms underlying direction selectivity, from neurons to circuits, is essential for understanding how we perceive our environment. Here, we report that a subtype of primary somato-sensory neurons, Aδ-LTMRs, are tuned to the direction of hair deflection as a result of developmental mechanisms dictating morphological features unique to this LTMR subtype. Aδ-LTMRs respond more strongly to hair deflection in the R direction than in the C direction. This tuning property results from polarization of Aδ-LTMR lanceolate endings, which are concentrated on the caudal side of hair follicles. BDNF from epithelial cells signaling through TrkB expressed in Aδ-LTMRs directs development of their lanceolate ending morphological polarization. Elimination of BDNF in hair follicle epithelial cells leads to loss of both morphological polarization and direction-selective tuning. Thus, polarized expression of BDNF in epithelial cells on the caudal sides of hair follicles underlies direction-selective tuning of Aδ-LTMRs.

Polarized expression in caudally located hair follicle epithelial cells is a distinguishing feature of BDNF because the related neurotrophic factor NT3, which is also transcribed in a subset of hair follicle epithelial cells, is expressed in a pattern that is not polarized. NT3 is expressed in a circumferential manner, in epithelial cells surrounding the hair follicle. While the polarized expression of BDNF in hair follicle epithelial cells is crucial for Aδ-LTMR

ending polarity, and thus the direction-selective tuning property that is unique to this LTMR subtype, it is dispensable for maturation and survival of these neurons. In contrast, mesenchyme-derived BDNF is essential for the general development of Aδ-LTMRs. Thus, distinct sources of BDNF contribute to different aspects of Aδ-LTMR development and function. Key to understanding the establishment of Aδ-LTMR lanceolate ending polarization, and thus direction selectivity of Aδ-LTMRs, is elucidating the mechanism of polarized BDNF transcription in hair follicle epithelial cells. It is possible that the same cues that govern polarization of other hair follicle features, such as the location of sebaceous glands and a variety of molecular markers, also control polarized expression of BDNF and thus Aδ-LTMR lanceolate ending polarization and their direction selectivity to hair deflection.

Of the many questions pertaining to direction-selective Aδ-LTMRs, a most intriguing one is how these ultrasensitive mechanosensory neurons respond preferentially to deflection of individual hairs in the R direction compared to the C direction. Aδ-LTMR longitudinal lanceolate endings associate with the caudal sides of hair follicles. Each neuron's cutaneous projection branches within the skin to innervate on average 35 awl/auchene and zigzag hair follicles, with 10–20 finger-like lanceolate endings closely associated with each follicle. Surrounding each lanceolate ending are processes of terminal Schwann cells (TSCs), which together with the lanceolate ending and hair follicle epithelial cells constitute “lanceolate complexes.” Ultra-structurally, each lanceolate complex exhibits gaps or openings in which the Aδ-LTMR axonal membrane on the side facing the hair follicle epithelial cell is exposed, forming a small, ∼80–90 nm protrusion extending between TSC processes, and these lanceolate ending protrusions lie within close proximity to the hair follicle epithelial cell basal lamina (Li and Ginty, 2014). Moreover, fine filament-like structures emanate from hemi-desmosomes positioned along the outer membranes of hair follicle epithelial cells, and these filaments, or putative tethers, extend through the basal lamina and appear to come in direct contact with the plasma membranes of both LTMR lanceolate axonal ending protrusions and TSCs (Li and Ginty, 2014). Because in vitro findings implicate tethers emanating from primary mechanosensory neurons as essential for mechanotransduction (Chiang et al., 2011), we previously speculated that the filamentous connections between epithelial cells and LTMR membranes mediate mechanotransduction in vivo, transducing hair deflection into lanceolate axon depolarization and LTMR excitation. Such a function of putative lanceolate complex tethers may thus be analogous to that of the tip links that extend between stereocilia of mechanosensory hair cells of the inner ear and that mediate mechanotransduction in the auditory and vestibular systems. If the LTMR-hair follicle tether model is indeed correct, then deflection of hairs in the R direction would be expected to pull on most putative tethers connecting hair follicle epithelial cells and Aδ-LTMR lanceolate axon membranes, leading to excitation of the Aδ-LTMR. Hair deflection in the C direction, on the other hand, would be expected to relax most putative tethers thus failing to open mechanically sensitive channels in Aδ-LTMR lanceolate axonal membranes. As such, enrichment of lanceolate complexes on the caudal side of hair follicles would underlie a greater sensitivity to hair deflection in the R direction than in the C direction. Identification of the protein composition of the putative epithelial cell-to-LTMR tether would enable ablation experiments that could test this and related ideas for hair deflection-LTMR

mechanotransduction and thus provide understanding of the mechanism of Aδ-LTMR direction selectivity.

In addition to the somatosensory system, direction selectivity is a key feature of other sensory systems, notably the auditory and visual systems. In the visual system, DS-RGCs report on the movement of images across their receptive fields. A morphological basis of direction selectivity for at least three DS-RGC subtypes was revealed by the discovery that their dendrites are polarized in the orientation of their preferred direction of movement of visual stimuli (Kim et al., 2008; Vaney et al., 2012). Thus, at least some DS-RGC subtypes and, as described here, Aδ-LTMRs have a morphological basis for their direction-selective tuning properties. In another noteworthy parallel, the central representations of peripheral receptive fields are topographically organized in both the visual and somatosensory systems. Retinotopic and somatotopic organization of the central representations of peripheral receptive fields may underlie direction selectivity in these sensory systems by virtue of temporal contrasts between the activities of peripheral neurons whose receptive fields are adjacent or in close proximity to one another. And yet, as reported here for the somatosensory system and previously for the visual system, both systems also have individual peripheral components, Aδ-LTMRs and DS-RGS, that are themselves tuned to the direction of stimulus movement. Are topographic maps and direction-selective peripheral units both employed for the central representation and interpretation of direction selectivity? Retinotopy- and somatotopy-based mechanisms for computing the direction of stimulus movement would necessarily rely on temporal contrasts of the spiking of two or more neurons with nonoverlapping receptive fields. On the other hand, in the somatosensory system, we find that Aδ-LTMRs can encode information about the direction of movement of a single hair. Thus, somatotopy-based computations and the direction-selective information coded by individual Aδ-LTMRs are distinct and may play complementary roles in perception of the direction of stimulus movement across hairy skin. Aδ-LTMRs may enhance the contrast of somatotopy-based computations that are driven by neurons that need not be direction-selective themselves, such as Aβ RA-LTMRs. Additionally, Aδ-LTMRs may report on the direction of hair deflection when all hairs on a skin area are simultaneously stimulated in the same direction, for example during exposure to a gentle breeze. In this scenario, all Aδ-LTMRs innervating the stimulated area are expected to respond in a similar manner. For this particular example, a somatotopy-based mechanism would lack temporal contrast between individual neuronal responses and therefore may be ineffective in reporting directionality of the stimulus. On the other hand, individually tuned, direction-selective Aδ-LTMRs are predicted to report on directionality independent of contrast between neurons having adjacent receptive fields and may therefore contribute to the perception of direction-selective movement of hairs under such a condition. To test these and related ideas, it will be important to establish the relative contributions of Aδ-LTMR direction selectivity to the perception of hair deflection direction and object movement across hairy skin.

How is direction-selective information, extracted from the skin by Aδ-LTMRs, conveyed to the brain? Insights into this question may be gleaned from studies of other sensory systems, and we again turn to direction-selective circuits of the visual system for analogy. DS-RGCs and non-direction-selective RGCs project to distinct regions of the thalamus, where higher

order neurons then project to distinct layers of visual cortex (Cruz-Martín et al., 2014). Thus, processing of direction-selective visual information and other visual information, such as light contrast, occurs at least in part via distinct brain circuitries. In the somatosensory system, many neurons of primate somatosensory cortex, representing both glabrous and hairy skin, are tuned to the direction of stimulus movement across the skin (Costanzo and Gardner, 1980; Hyvärinen and Poranen, 1978; Whitsel et al., 1972, 1978). Moreover, perception of the direction of stimulus movement across the skin requires the integrity of the dorsal column pathway (Bender et al., 1982; Vierck, 1974), a major ascending tract that contains primary branches of Aβ-LTMRs and postsynaptic (indirect) dorsal column pathway neurons, both of which terminate in the dorsal column nuclei of the brainstem, the gracile and cuneate nuclei. Indeed, while disruption of the dorsal column eliminates the perception of direction of tactile stimulus movement in primates, dorsal column lesions alone have little or no impact on the general detection of stimulus motion. Lesions of both the dorsal columns and the dorso-lateral funiculus disrupt perception of both stimulus direction and motion (Vierck, 1974; Wall and Noordenbos, 1977). We find that Aδ-LTMR central projections terminate in lamina IIiv-III of the dorsal horn, however, unlike Aβ-LTMR subtypes, Aδ-LTMRs do not have a branch that ascends the dorsal column. Therefore, we speculate that direction-selective information about hair deflection, at least that which is extracted by Aδ-LTMRs, is processed in the spinal cord dorsal horn and subsequently conveyed to the brain via postsynaptic dorsal column neurons comprising the indirect dorsal column pathway. The identity of postsynaptic partners of Aδ-LTMRs in the dorsal horn, how Aδ-LTMR direction-selective information is conveyed from the dorsal horn to the brain, and the relative contributions of Aδ-LTMRs to direction-selective tuning properties of neurons in the neocortex and to the perception of direction of object movement across hairy skin await findings of future interrogation of these fascinating neurons.

Experimental Procedures

Mouse Lines

The *TrkB*^{tauEGFP} (Li et al., 2011), *TH*^{CreER} (Badea et al., 2009), *Ret*^{CreER} (Luo et al., 2009), T-Cre (Perantoni et al., 2005), *Wnt1-Cre* (Danielian et al., 1998), *K5-Cre* (Ramirez et al., 2004), *Rosa26-TdTomato* (strain Ai9; Jackson Laboratory), *Rosa26-iAP* (Badea et al., 2009), *BDNF-loxp* and *BDNF-LacZ* (Gorski et al., 2003), *NT3-LacZ* (Fariñas et al., 1994), and *NT4-LacZ* (EUCOMM) mouse lines have been described. *Split*^{Cre} mice express Cre recombinase in Aβ RA-LTMRs and will be described elsewhere. *TrkB*^{CreER} mouse line generation is described in Extended Experimental Procedures.

Electrophysiological Recordings

Intracellular electrophysiological recordings from TrkB^{CreER}-labeled skin sensory neurons were obtained in adult animals, using either ex vivo somatosensory system preparations and DRG neurons innervating dorsal back skin or in vivo preparations and trigeminal ganglion neurons innervating the face. Generation of the ex vivo cutaneous somatosensory system preparation used in the present studies has been described in detail (Li et al., 2011; Woodbury et al., 2001). Generation of in vivo adult mouse preparation was modified from

procedures detailed elsewhere (Boada and Woodbury, 2007), as described in Extended Experimental Procedures.

Receptive Field Analyses

To investigate the response properties of LTMRs to directional movement of hairs, fine-grained receptive field (RF) analyses were conducted by characterizing the sensitivity of cells to controlled movements of hairs located in multiple spots throughout the RF; in many cases this amounted to systematic mapping of the RF in a hair-by-hair manner. To achieve controlled directional movement of hairs, a variety of customized probes were tested before settling on a forked, comb-like device that allowed us to unambiguously isolate and trap individual hairs under direct visual observation at high magnification. This probe consisted of two 0.1 mm diameter minuten pins glued together in parallel, leaving ∼0.5 mm of the tapered tips exposed, the latter bent at ∼90° to form a miniaturized two-tined rake (see Figure S2F). This rake was oriented orthogonally to the skin and against the grain using a manual micromanipulator equipped with precision lead screws (100 TPI). Hairs normally laid flat, and once trapped, erected to an ∼45° angle prior to initiating a series of alternating movements in the rostrocaudal (RC) and dorsoventral (DV) planes. These movements were repeated at ∼1 Hz and displaced the probe ∼0.5 mm at a rate of ∼2 mm/s. To control for the possibility that responses in the cell might reflect slippage of the hair shaft in the yoke of the probe during movement (i.e., sensitivity to vibration produced by cuticular irregularities along the hair shaft), we used a different probe tip in some experiments that was fashioned from a single minuten pin coated in glue from sticky mouse traps (Stick-Em, JT Eaton); this glue-tipped probe could be affixed securely to individual hairs to dampen or completely prevent potential microvibrational influences during movement. The numbers of spikes elicited by movements in each direction were counted and averaged across each spot tested in the receptive field; responses to movements in different directions were compared using either Student's t tests and/or ANOVAs with Tukey's post hoc corrections (Origin Pro 8).

Histological Analyses

Immunohistochemistry of tissue sections, whole mount immunohistochemistry, in situ hybridization, whole mount PLAP staining of the skin and spinal cord, and LacZ staining were done using standard procedures (see Extended Experimental Procedures for details).

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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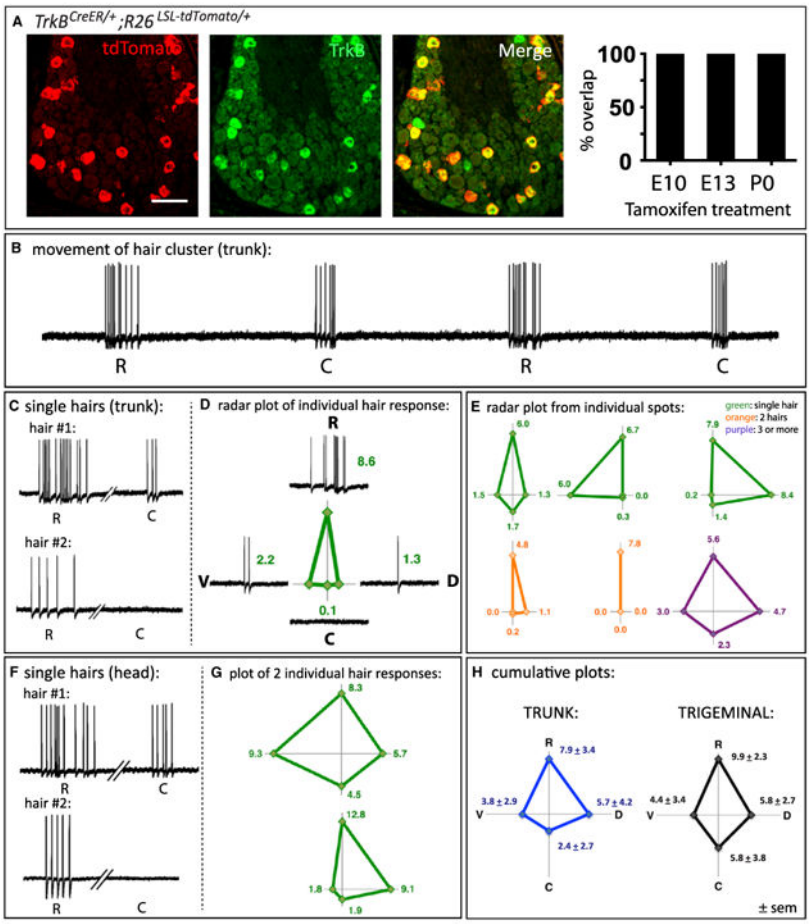

Figure 1. Aδ-LTMRs Exhibit Direction-Selective Tuning to Hair Follicle Deflection

(A) Pregnant *TrkB*$^{CreER/+}$;*Rosa26*$^{LSL\text{-}tdTomato/+}$ mice were treated with tamoxifen (3 mg at either E10, E13, by oral gavage or a single intraperitoneal [i.p.] injection of 1mg at P0). Double fluorescence in situ hybridization for tdTomato (red) and TrkB (green) on thoracic DRG sections from P21 mice reveals that virtually all tdTomato+ DRG neurons are TrkB^{+} (100%, 194/194). A small subset of TrkB+ cells observed by in situ hybridization (green) are not labeled with the TrkB^{CreER} labeling strategy (3/27 in this particular image). Scale bar represents 100 um.

(B) Intracellular ex vivo electrophysiological recordings from a *TrkB*^{GFP}-labeled Aδ-LTMR reveal selectivity of response to repetitive stimulation of a small cluster of hairs in the rostrocaudal direction; note the consistently greater response elicited by deflections in the rostral (R) versus caudal (C) direction.

(C) Examples of responses to deflection of three different single hairs in the receptive fields of Aδ-LTMRs in *K5*$^{Cre/+}$; *BDNF*$^{flox/+}$; *TrkB*$^{GFP/+}$ mice.

(D) Vector plot of average responses to multiple deflections of a single hair in the four cardinal directions.

(E) Examples of different vector plots elicited by movements of hairs in the receptive fields of Aδ-LTMRs in $K5^{Cre/+}$; $BDNF^{flox/+}$; $TrkB^{GFP/+}$ mice (green, single hairs; orange, hair doublets; purple, cluster).

(F) In vivo electrophysiological recordings of Aδ-LTMRs in trigeminal ganglia of $TrkB^{CreER/+}$;$Rosa26^{LSL\text{-}tdTomato/+}$ mice showing two different examples of responses elicited by controlled deflections of single hairs.

(G) Examples of vector plots of average responses to multiple deflections of single hairs in the four cardinal directions in trigeminal Aδ-LTMRs in vivo.

(H) Cumulative vector plot of all individual locations analyzed throughout the receptive fields of trunk and trigeminal Aδ-LTMRs in vivo, revealing a similar tuning for rostral deflections of hairs as in trunk DRG. Shown are the means ± SD. Scale bars represent 25 mV (B, C, and G), 250 ms (B and C), and 100 ms (G). See also Figures S1, S2, and S3.

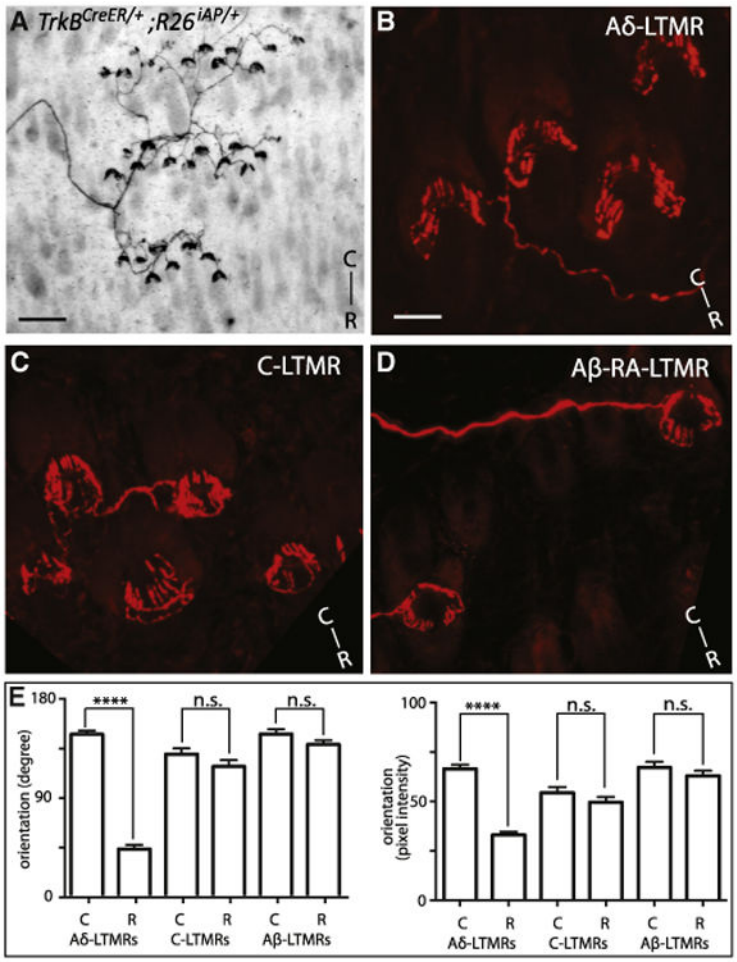

Figure 2. Aδ-LTMRs Endings Are Polarized around Hair Follicles

(A) Visualization of Aδ-LTMR peripheral receptive fields. *TrkB*$^{CreER/+}$;*Rosa26*$^{iAP/+}$ mice were treated with tamoxifen (1 mg at E13 or P25, by oral gavage) and whole mount, alkaline phosphatase preparation performed on skin of P21 or P40–P50 animals. Each Aδ-LTMR was found to arborize and form longitudinal lanceolate endings associated an average of 35 zigzag and awl/auchene hair follicles. Scale bar represents 100 um.

(B) Aδ-LTMR longitudinal lanceolate endings are polarized toward the caudal side of hair follicles. Whole mount immunohistochemical staining showing Aδ-LTMR longitudinal lanceolate endings more densely populated on the caudal (top of the image) side (note: *TrkB*$^{CreER/+}$;*Rosa26*$^{LSL\text{-}tdTomato/+}$ mice treated with 1 mg tamoxifen at E13; n = 4). Scale bar represents 20 μm.

(C and D) There is no obvious polarization of C-LTMR and Aβ RA-LTMR longitudinal lanceolate endings. Whole mount immunohistochemical staining of sparsely labeled C-LTMR lanceolate endings in *TH*$^{CreER/+}$;*Rosa26*$^{LSL\text{-}tdTomato/+}$ mice treated with 2 mg tamoxifen/day at P13–P15, n = 3. Whole mount immunohistochemical staining of sparsely labeled Aβ RA-LTMR lanceolate endings in *Ret*$^{CreER/+}$;*Rosa26*$^{LSL\text{-}tdTomato/+}$ mice treated with 1 mg tamoxifen/day at E10 and E11 (n = 3).

(E) Quantification of orientation/morphological properties of Aδ-, C-, and Aβ RA-LTMR longitudinal lanceolate endings. Shown are means ± SEM. Left: illustration of central angle measurement to compare the density of lanceolate endings on caudal versus rostral side of the hair follicle; Right: summary of pixel intensity quantification by the ImageJ software. Lanceolate ending polarity was assessed by central angle and pixel intensity measurement on mice with sparsely labeled Aδ-, C-, and Aβ RA-LTMRs, respectively ($n = 3$). *$p < 0.05$. ***$p < 0.001$.

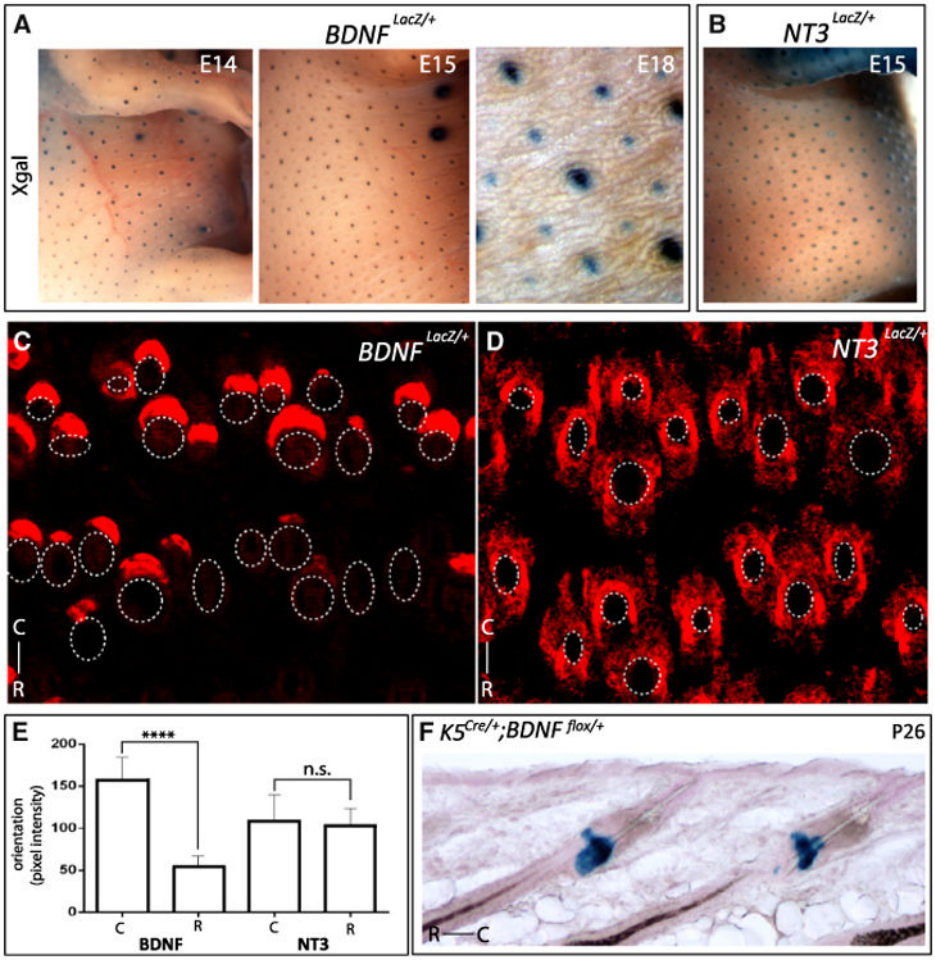

Figure 3. BDNF Expression Is Highly Polarized to the Caudal Side of Hair Follicles

(A and B) Embryonic *BDNF*$^{LacZ/+}$ and *NT3*$^{LacZ/+}$ tissue was examined by whole mount Xgal staining. Robust BDNF-LacZ and NT3-LacZ expression was detected in developing hair follicles throughout all three stages of hair follicle morphogenesis.

(C and D) Immunostaining of βgal (red) on back hairy skin sections from *BDNF*$^{LacZ/+;}$ and *NT3*$^{LacZ/+}$ P7 mice shows that BDNF-LacZ expression is polarized while NT3-LacZ expression is not.

(E) Summary of pixel intensity quantification by the ImageJ software. LacZ expression polarity was assessed by pixel intensity measurement on P7 mice. Shown are means ± SEM. ****$p < 0.001$.

(F) Xgal staining on *K5*$^{Cre/+}$*; BDNF*$^{flox/+;}$embryos shows that hair follicle-derived BDNF originates in epithelial cells.

See also Figure S4.

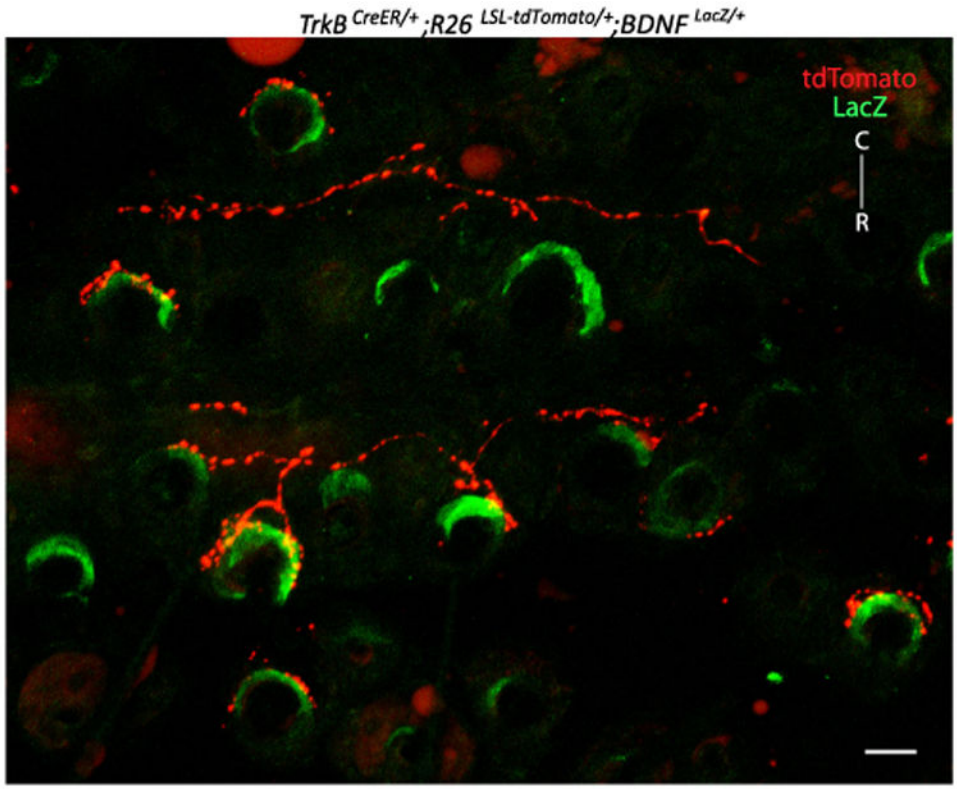

Figure 4. BDNF Expression Is Temporally Coincident with and Occurs in Close Apposition to Developing Aδ-LTMRs Longitudinal Lanceolate Endings

In hairy skin sections from P5 *TrkB*$^{CreERT2/+}$;*Rosa26*$^{LSL\text{-}tdTomato/+}$;*BDNF*$^{LacZ/+}$ mice, Aδ-LTMR endings axonal endings are labeled with tdTomato fluorescence (red) and BDNF is labeled by βgal immunostaining (green). Cre recombinase was induced by administration of 1 mg tamoxifen at E13 (oral gavage, n = 2). Note that postnatal, BDNF-producing epithelial cells are in close apposition to Aδ-LTMR endings. Scale bar represents 30 μm.

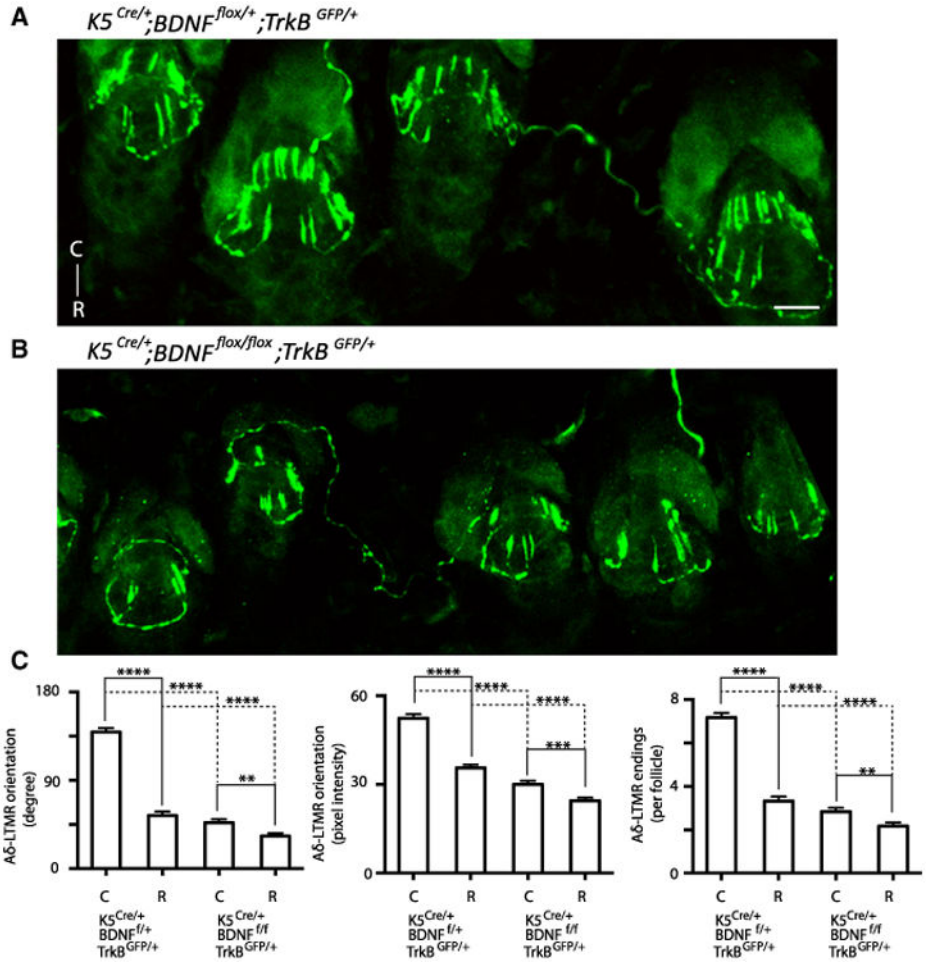

Figure 5. BDNF Expression in Hair Follicle Epithelial Cells Is Required for Polarization of Aδ-LTMR Endings

(A and B) Whole mount skin staining in *K5*$^{Cre/+}$*; BDNF*$^{flox/+}$ *TrkB* $^{GFP/+}$ and *K5*$^{Cre/+}$*; BDNF* $^{flox/flox}$*;TrkB*$^{GFP/+}$ mice. Aδ-LTMR lanceolate endings (GFP, green) are polarized on the caudal side of hair follicles in littermate control *K5*$^{Cre/+}$*; BDNF*$^{flox/+}$*; TrkB*$^{GFP/+}$ mice (A). Loss of Aδ-LTMR lanceolate ending polarity in *K5*$^{Cre/+}$*; BDNF*$^{flox/flox}$*;TrkB*$^{GFP/+}$ mice (B) (endings associated with 106 hair follicles; n = 2 mice).

(C) Aδ-LTMR lanceolate ending polarity is dramatically reduced in *K5*$^{Cre/+}$*; BDNF*$^{flox/flox}$ mice. Lanceolate ending polarity was assessed by central angle and pixel intensity measurement and quantification of lanceolate endings in *K5*$^{Cre/+}$*; BDNF*$^{flox/flox}$*;TrkB*$^{GFP/+}$ and littermate control *K5*$^{Cre/+}$*; BDNF*$^{flox/+}$*; TrkB*$^{GFP/+}$ mice. 106 hair follicles were quantified for each genotype (n = 2). Scale bar represents 20 μm.

See also Figure S5.

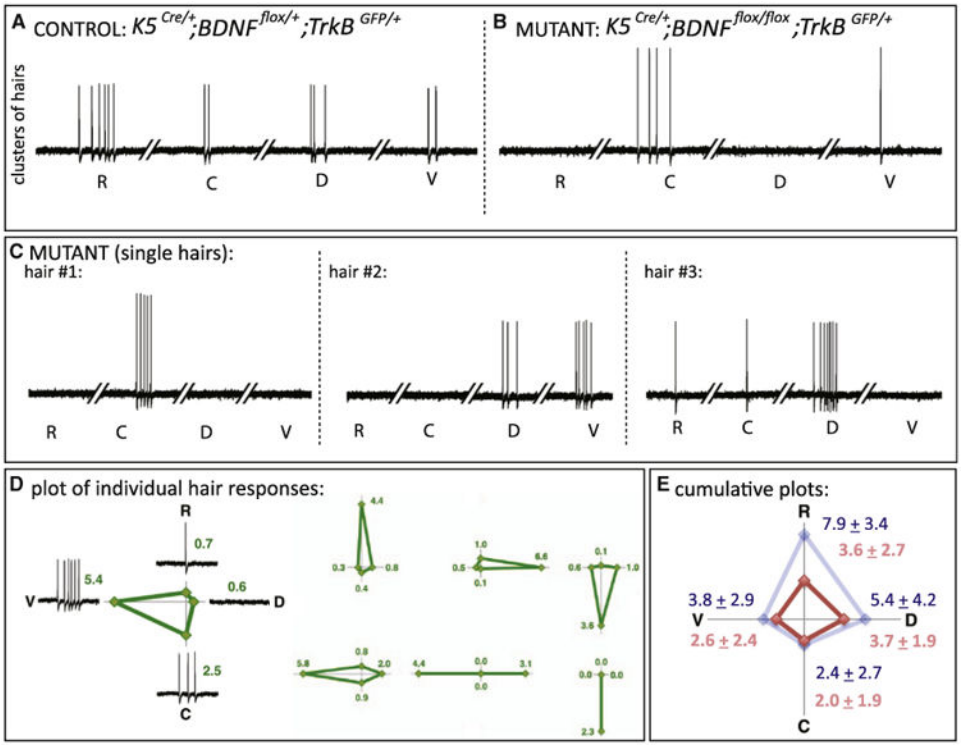

Figure 6. BDNF Expression in Hair Follicle Epithelial Cells Is Required for Direction-Selective Tuning of Aδ-LTMRs

(A and B) Ex vivo intracellular recordings of Aδ-LTMR responses elicited by deflecting a small cluster of hairs in (A) *K5*$^{Cre/+}$*; BDNF*$^{flox/+}$*; TrkB*$^{GFP/+}$ control mice, and (B) *K5*^{Cre}, *BDNF*$^{flox/flox}$*; TrkB*$^{GFP/+}$ mutant mice; note the selective tuning in the opposite, caudal direction in the latter.

(C) Examples of responses to controlled deflection of three different single hairs in the receptive fields of Aδ-LTMRs in *K5*^{Cre}*; BDNF*$^{flox/flox}$*; TrkB*$^{GFP/+}$ (mutant) mice.

(D) Vector plot of average responses to multiple deflections of a single hair in the four cardinal directions and examples of different vector plots elicited by movements of single hairs in the receptive fields of Aδ-LTMRs in *K5*^{Cre}*; BDNF*$^{flox/flox}$*; TrkB*$^{GFP/+}$ mice. Note the random, whorled orientations compared to controls (Figure 1).

(E) Shown are the means ± SD of spike numbers obtained from recordings of six neurons of each genotype, with responses to deflections of hairs in 60 and 45 individual locations from mutant and wild-type mice, respectively. Cumulative vector plot of all individual locations analyzed throughout the receptive fields of Aδ-LTMRs in *K5*^{Cre}*; BDNF*$^{flox/flox}$*; TrkB*$^{GFP/+}$ mice (orange); note that the average response of all hairs combined lacks the direction-selective tuning observed overall in Aδ-LTMRs from control mice (blue). The latter represent replotted data from the ex vivo recording results from control mice, shown in Figure 1H.

See also Figure S6.