Merkel Cells Are Essential for Light-Touch Responses

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The peripheral nervous system detects different somatosensory stimuli, including pain, temperature, and touch. Merkel cell-neurite complexes are touch receptors composed of sensory afferents and Merkel cells. The role that Merkel cells play in light-touch responses has been the center of controversy for over 100 years. We used Cre-loxP technology to conditionally delete the transcription factor Atoh1 from the body skin and foot pads of mice. Merkel cells are absent from these areas in Atoh1CKO animals. Ex vivo skin/nerve preparations from Atoh1CKO animals demonstrate complete loss of the characteristic neurophysiologic responses normally mediated by Merkel cell-neurite complexes. Merkel cells are therefore required for the proper encoding of Merkel receptor responses, suggesting that these cells form an indispensable part of the somatosensory system.

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Different qualities of touch are encoded by discrete touch receptors, each with distinctive coding properties (1–3). One form of light touch important for tactile discrimination of shapes and textures is mediated by Merkel cell-neurite complexes, which exhibit a characteristic response to light skin indentation (4, 5). Merkel cell-neurite complexes are composed of nerve fibers associated with Merkel cells, an enigmatic skin cell population first described in enigmatic skin cell population first described in mannnnian skin, Merkel cells are normally found in whisker follicles of the face, specialized epithelial structures of the hairy skin called touch domes, and epidermal invaginations of the plantar foot surface called rete ridges (7). Merkel cells have been proposed to be the sensory receptor cells of the complexes because they form synaptic contacts with somatosensory afferents (8, 9); however, studies that indirectly tested this model have yielded conflicting results (10–16).

Atoh1 is a basic helix-loop-helix transcription factor expressed by Merkel cells in all areas of the skin (17). Atoh1 null mice die within minutes of birth, which prevents a detailed assessment of nonlethal phenotypes resulting from deletion of the gene. We used the Hoxb1Cre allele (18), which is expressed throughout the epidermis and dermis of body skin, but not head skin (Fig. 1, A to A′), to delete a floxed allele of Atoh1 (Atoh1Flox) (19) in transgenic mice (20). Conditional knockout (Atoh1CKO) animals were born in the expected Mendelian ratio, but roughly 50% of these animals died within 24 to 36 hours of birth.

The overall structure of the touch dome, including the palisading epithelium and location of the guard hair, was preserved in Atoh1CKO animals (Fig. 1, B and C). Xgald staining was found in touch domes and foot pads of heterozygous Atoh1Flox mice but not Atoh1CKO mice (Fig. 1, B′ and C′). To determine whether Merkel cells were present, we

Fig. 1. Hoxb1Cre abolishes Atoh1 expression in the body but not the face.

Whelworm X-gal staining of E16.5 embryos revealed β-galactosidase expression driven from the ROSA26R allele (A to A′) or Atoh1loxP (B to C′). (A) Hoxb1Cre, ROSA26R embryo. Hoxb1Cre expression is absent from the majority of the head. Boxes denote regions shown in (A) and (A′). (A′ and A′′) Body skin and whisker pad, respectively, from a Hoxb1Cre, ROSA26R embryo counterstained with nuclear fast red. Staining is present throughout the epidermis and dermis of the body skin, whereas virtually no staining is seen in the whisker pad. The bracket in (A′) denotes a developing touch dome. WF, whisker follicle. (B) Atoh1loxP embryo showing β-galactosidase expression in the whisker pad (bracket) and touch domes of the skin. The box denotes the region shown in (B′). (B′) Atoh1loxP; Atoh1LacZ/flox embryo skin showing staining in individual touch domes. (C) Atoh1CKO embryo skin showing β-galactosidase expression driven from the Atoh1 locus in the whisker pad (bracket), but absent from touch domes of the skin (box). (C′) Atoh1CKO embryo body skin. Touch domes are present but lack the WT staining pattern. Scale bars, 5 mm [(A), (B), and (C)]; 400 μm [(B′) and (C′)]; 100 μm [(A′) and (A′′)].
used immunocytochemistry to compare the expression pattern of keratin 8, an intermediate filament protein specifically expressed by Merkel cells in adult mammalian skin (23, 24), with that of β-galactosidase protein expression driven from the Atoh1\textsuperscript{loxP}\textsuperscript{Z} locus (Fig. 2). Both proteins were expressed by Merkel cells in all regions of Atoh1\textsuperscript{floxed} animals but were absent throughout the body of Atoh1\textsuperscript{CKO} animals, except for the whisker pads where Hoxb1\textsuperscript{Cre} is not expressed. We also found that VGLUT2 and Rab3c, two synaptic vesicle proteins that robustly label Merkel cells (8, 25, 26), were absent from the epidermis of body skin and foot pads (Fig. 3, A and B, and fig. S2) but were present in whisker pads of Atoh1\textsuperscript{CKO} animals. We confirmed these findings by examining more than 100 touch domes, 16 foot pads, and 8 whisker pads from four adult Atoh1\textsuperscript{CKO} mice. These results differ from a previous report from our group suggesting that Merkel cells are present in Atoh1-null embryos (17). That study was limited by the necessity of examining prenatal mice, because Atoh1-null animals die within minutes of birth secondary to respiratory failure (22). Here, our use of conditional knockout animals enabled us to examine specific knockout animals in fully developed skin and to show Merkel cell loss in Atoh1\textsuperscript{CKO} mice. To our knowledge, Atoh1 is the first gene shown to be necessary for the specification of Merkel cells. Our data also demonstrate that Merkel cells are not necessary to specify or maintain touch dome ultrastructure, as guard hairs and the overlying keratinocytes appear completely normal in the hairy skin of Atoh1\textsuperscript{CKO} mice.

Crescent-shaped clusters of Merkel cells are normally found within each touch dome, where they are innervated by a single sensory afferent that expresses neurofilament 200 (NF200) in large- and medium-sized branches and VGLUT2 in terminal branches (8, 26) (Fig. 3A). Innervation of touch domes was present in wild-type (WT) and Atoh1\textsuperscript{CKO} animals, as revealed by NF200 immunocytochemistry (Fig. 3, A and B). However, there was exuberant branching of the VGLUT2-positive, NF200-negative terminal ends of touch dome afferent fibers of Atoh1\textsuperscript{CKO} animals (Fig. 3B). We confirmed this observation using in vivo subcutaneous injections of the styryl dye FM 1-43. FM 1-43 strongly labels sensory cells, including Merkel cells, and their afferent fibers in vivo (27) in WT mice, permitting whole-mount analysis of Merkel cell-neurite complex structure (Fig. 3C). Afferent terminal branches were labeled in the touch domes of Atoh1\textsuperscript{CKO} animals, despite the absence of Merkel cells (Fig. 3D). Both of these methods demonstrated that touch domes in Atoh1\textsuperscript{CKO} animals display excessive terminal branching compared with WT animals. These data suggest that, although Merkel cells are not necessary for the development or maintenance of touch dome innervation, they play a role in the acquisition of the typical terminal arborization pattern of touch dome afferents. Several neurotrophins have been implicated in the development and maintenance of Merkel cell innervation (28, 29). Our data also demonstrate that Merkel cells cannot be the primary source of these trophic factors.

Many different afferent somatosensory fiber types innervate the skin (30). These fibers can be grouped by conduction velocity into three broad categories: Aβ, Aδ, and C fibers (table S1). Nociceptors and temperature receptors are primarily of the Aδ and C subtypes, whereas light-touch sensation is mediated by Aβ fibers. The Aβ fiber subclass can be further subdivided by the adaptation characteristics of the fibers: Slowly adapting type I (SAI) fibers innervate Merkel cell-neurite complexes (5), SAI fibers are thought to innervate Ruffini corpuscles, and rapidly adapting (RA) fibers innervate Meissner and Pacinian corpuscles (1). Each of these subclasses is important for detecting a specific form of touch (1). Together with the presence of touch dome innervation, the absence of Merkel cells in Atoh1\textsuperscript{CKO} animals provided the perfect opportunity to test whether Merkel cells are required for mechanotransduction by their innervating nerve fibers.

The overall population of cutaneous afferent receptors is normal in Atoh1\textsuperscript{CKO} animals (Fig. 3, J to L). We applied electrical and mechanical stimuli to the epidermal surface of ex vivo skin/saphenous nerve preparations from WT and Atoh1\textsuperscript{CKO} animals and simultaneously recorded extracellular responses from teased afferent fibers (Fig. 3, E to M). There were no differences between WT and Atoh1\textsuperscript{CKO} mice in the mechanical thresholds (Fig. 3J), conduction velocities (Fig. 3K), or proportions (Fig. 3L) of touch-sensitive fibers (P > 0.1 by Mann-Whitney U test).

We next focused specifically on the Aβ fiber population. The distribution of Aβ subtypes revealed a conspicuous loss of SAI responses among slowly adapting Aβ afferents in Atoh1\textsuperscript{CKO} animals [n = 0 out of 27 (0/27) afferents] compared with WT animals (n = 8/39 afferents) (Fig. 3M). We also observed a proportional expansion of other Aβ afferent subtypes (20) in Atoh1\textsuperscript{CKO} mice. These data are consistent with a complete loss of mechanosensitive SAI fibers.
Thus, canonical SAI responses elicited by touch require Merkel cells. It is possible that touch dome afferents lacking Merkel cells remain intrinsically touch sensitive but possess different electrophysiological properties. For example, they could be represented in the “ambiguous” class (fig. S1); however, they are unlikely to constitute the whole population because we observed similar responses in WT mice.

For more than a century, neurobiologists have postulated that Merkel cells are responsible for the specialized coding properties that allow their afferent nerves to resolve fine spatial details. Our genetic knockout approach has allowed us to directly test this hypothesis and to demonstrate that Merkel cells are essential for these responses. Because Merkel cells fail to develop in Atoh1CKO mice, a key question that remains is whether Merkel cells, somatosensory neurons, or both are sites of mechanotransduction in the skin. Selective and acute control of Merkel cell signaling will be necessary to determine whether Merkel cells act as sensory receptor cells or serve another role in touch.

References and Notes

20. Materials and methods are available as supporting material on Science Online.
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Supporting Online Material

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Materials and Methods
Figs. S1 and S2
Table S1
References

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