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Merkel Cells in Somatosensation

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Abstract

Merkel cells are rare epidermal cells whose function in the skin is still debated. These cells localize to highly touch-sensitive areas of vertebrate epithelia, including palatine ridges, touch domes and finger tips. In most cases, Merkel cells complex with somatosensory afferents to form slowly adapting touch receptors; it is unclear, however, whether mechanosensory transduction occurs in the Merkel cell, the somatosensory afferent or both. Classic anatomical results suggests that Merkel cells are sensory cells that transduce mechanical stimuli and then communicate with sensory afferents via neurotransmission. This model is supported by recent molecular, immunohistochemical and physiological studies of Merkel cells *in vitro* and in intact tissues. For example, Merkel cells express essential components of presynaptic machinery, including molecules required for release of the excitatory neurotransmitter glutamate. Moreover, Merkel cells *in vitro* and *in vivo* are activated by mechanical stimuli, including hypotonic-induced cell swelling. Although these findings support the hypothesis that Merkel cells are sensory receptor cells, a definitive demonstration that Merkel cells are necessary and sufficient to transduce touch awaits future studies.

Keywords

epidermis; mechanotransduction; somatosensory; touch

Somatosensory transduction in the oral cavity

Along with the senses of taste and olfaction, the somatic senses of touch, thermoreception and pain (nociception) contribute to our ingestion and enjoyment of food. In the oral cavity, somatosensory neurons of the trigeminal, glossopharyngeal and vagal nerves encode information about the chemical and physical qualities of foodstuffs (Simon *et al.*, 2006). For example, polymodal nociceptors innervating the tongue and palate are essential for detecting the pungent tastants found in chili peppers and wasabi (Caterina *et al.*, 2000; Bautista *et al.*, 2006; Kwan *et al.*, 2006), as well as cooling agents such as menthol (Daniels and McKemy, 2007). In addition to these chemical irritants, polymodal nociceptors are activated by noxious temperatures, harsh mechanical stimuli and inflammatory mediators.

The oral epithelium is also innervated by mechanosensitive neurons that monitor the positions of the tongue and foodstuffs during chewing. In the palate, at least four subtypes of somatosensory receptors have been classified as touch receptors based on morphological similarities to touch-sensitive neurons that innervate the skin. One receptor subtype, the Merkel cell-neurite complex, is particularly abundant in palatine ridges (Tachibana *et al.*, 1997; Halata *et al.*, 1999; Nunzi *et al.*, 2004). Although the role of Merkel cell-neurite complexes in innocuous touch is well established, the function of the Merkel cell within the complex is still debated. Here, we present an historical overview of the discovery of somatosensory mechanoreceptors and review progress on the role of Merkel cells in somatosensation. This

manuscript is intended to complement excellent reviews of somatosensation in the oral cavity (Simon *et al.*, 2006) and Merkel cells across vertebrate species (Tachibana and Nawa, 2002; Halata *et al.*, 2003).

Somatosensory modalities

The mechanisms by which animals sense touch, temperature and pain have been the subject of scientific inquiry since at least the third century BCE, when Aristotle included touch in his five cardinal senses. Anatomical experiments in the nineteenth century revealed a remarkable assortment of sensory structures within the dermis and underlying tissues of the skin. Concurrently psychophysical experiments discovered the presence of sensory spots on the skin that were highly sensitive to specific stimulus modalities in humans (Hamann, 1995). This spatial localization of structure and function led von Frey and others to postulate the specificity theory of somesthesia – cutaneous senses are subserved by neurons that are specialized to respond to only one sensory modality (Boring, 1942).

The sensory modalities of somesthesia are innocuous touch, cool, warm and nociception. Which sensory modalities are mediated by the various somatosensory structures was the subject of much speculation: most sensory structures are not visible in living humans, and the inability to record the activity of sensory neurons in animals prevented definite attribution of individual sensory modalities to specific neuronal populations. Consequently, the strict labeled-line doctrine of somesthesia was controversial.

Physiological analysis of somatosensation awaited the development of single unit recordings of primary afferent fibers. In 1926, Adrian and Zotterman developed the first live, semi-intact nerve recordings of primary sensory afferents and their associated cutaneous targets (Adrian and Zotterman, 1926; Zotterman, 1939). They discovered individual afferents specifically activated by light touch, supporting von Frey's specificity theory. Subsequent work revealed the presence of specific thermosensitive and nociceptive afferents (Zotterman, 1939; Dodt and Zotterman, 1952; Hensel and Boman, 1960).

Given that different sensory modalities are mediated by labeled lines, one possibility is that the intensity of a given stimulus is encoded by firing rates of individual neurons. Mountcastle and colleagues tested this theory by recording from sensory afferents while applying quantitative indentations of the skin (Werner and Mountcastle, 1965). They described mechanically sensitive fibers responding to indentation of mechanosensitive touch domes with the following power function: $R = K \cdot S^n$ where R is the mean response number, K is a constant, S is the stimulus intensity measured as net skin indentation and the exponent n is a constant. Subsequent research has described a number of power functions that fit the stimulus-response relationship, depending on the type of mechanosensitive afferent measured and the response period monitored (Knibestöl, 1975). Mountcastle extended his work by recording similar mechanically induced stimulus-response functions in the spinal cord and the primary somatosensory areas of the brain, indicating that stimulus intensity is primarily encoded by the sensory afferent terminals and that transformations in central areas of the nervous system are linear (Mountcastle, 1967; Harrington and Merzenich, 1970). More recent research in the human hand revealed that different mechanoreceptors have varied stimulus-response functions despite a linear relationship between stimulus intensity and percept strength (Knibestöl and Vallbo, 1980). These data indicate, contrary to Mountcastle's hypothesis, a non-linear relationship between afferent firing and percept strength, indicating the presence of diverse non-linear transforms in higher brain regions.

Response profiles of mechanosensory afferents

Additional studies of somatosensory afferents in various vertebrates revealed five consistent stimulus-response profiles, which are characterized by their adaptive properties (Hamann, 1995). Rapidly adapting afferents fire bursts of action potentials to skin movement and vibration, but are insensitive to sustained skin deformation (Fig. 1). Slowly adapting afferents, by contrast, can continue to respond to sustained skin deformation for more than 30 min (Iggo and Muir, 1969). Both rapidly adapting and slowly adapting response profiles are further subdivided into two categories: type I and type II. Type I responses have receptive fields with small, sharp borders whereas type II responses have large, poorly defined receptive fields.

Rapidly adapting type I (RAI) afferents respond to bending of hairs, gentle rubbing of skin, and vibration (Brown and Iggo, 1967; 2630; Petit and Burgess, 1968; Iggo and Findlater, 1984). In the hand, RAI afferents are the most sensitive mechanoreceptors, responding to surface features as small as 2 μm . Rapidly adapting type II responses (RAII), which have been definitively correlated with Pacinian corpuscles (Iggo and Findlater, 1984; Bell *et al.*, 1994), respond most robustly to high frequency vibration (Hunt and McIntyre, 1960). Slowly adapting type I (SAI) afferents respond specifically to indentation of skin containing Merkel cell-neurite complexes (Iggo and Muir, 1969), and have the highest spatial resolution of the afferent response profiles. Slowly adapting type II (SAII) afferents, many of which are spontaneously active, respond more effectively to skin stretch than to indentation (Brown and Iggo, 1967; Leem *et al.*, 1993). In addition, many respond preferentially to specific orientations (Hamann, 1995). Finally, C-mechanoreceptors are rapidly adapting C-fibers that respond to both skin indentation and bending of hairs (Nordin, 1990).

Definitive correlation of afferent responses and sensory perception was not possible until the advent of primary afferent microneurography in awake humans. In this experimental paradigm, extremely thin electrodes record electrical impulses from neurons innervating the skin. Mechanical stimuli such as a pen point are applied to the hand, and single afferent responses associated with a tactile unit are identified. The electrode is then used to stimulate the single afferent fiber, and the subject reports the quality of sensation generated. Most of these studies have been performed in the hand, which has a high density of mechanosensitive afferents (Vallbo *et al.*, 1984; Johnson and Hsiao, 1992). Such studies, combined with traditional electrophysiological recordings in primates, indicate that rapidly adapting afferents encode skin motion. These afferents are integral to transmitting information related to low frequency vibration and the sensation of slip between skin and a grasped object (Johnson *et al.*, 2000). Microstimulation of RA afferents in humans has been described as a “wobbling” or “fluttering feeling” with very short electrical trains described as a “tap” (Vallbo *et al.*, 1984). RAI or PC afferents, which respond best to vibratory stimuli, are described, not surprisingly, by human subjects as a “vibration” sensation. Since SAI responses are more sensitive to forces tangential to the skin than indentation, they likely encode skin stretch. Microstimulation of SAI afferents has been described as percepts ranging from sustained lateral pulling of the skin to flutter (Vallbo *et al.*, 1984). By contrast, SAI afferents encode texture and small feature discrimination, and are necessary for fine tactile discrimination tasks, such as detecting Braille-like patterns (Johnson *et al.*, 2000). They are extremely sensitive to local deformations: a single Braille-like dot elicits 20 times more firing than a smooth surface (Phillips and Johnson, 1981). Microstimulation of single SAI afferents induced humans to feel a faint and uniform pressure, much like a “soft painting brush held tangentially against the skin” (Vallbo *et al.*, 1984).

Merkel cell-neurite complexes underlie SAI responses

In 1969, Iggo and Muir identified highly touch-sensitive spots in hairy skin, called touch domes, as the source of a slowly adapting response (Iggo and Muir, 1969). These structures are visible in depilated rodents and cats, and Iggo and Muir were able to characterize the response profile. They noted that SAI fibers are completely silent in the absence of mechanical stimulation of the touch dome. Sustained touch-dome indentation elicits a burst of action potentials followed by a slowly adapting response (Fig. 1). The sustained element of the response is characterized by an irregular firing pattern, with interspike intervals ranging from <20 to 220 ms. This feature, in particular, differentiates SAI responses from SAII responses, which have a highly regular sustained firing pattern. Iggo and Muir noted that the apex of the touch dome was highly sensitive to indentation, but firing rates dropped precipitously with indentation of the surrounding tissue. Measurement of conduction velocity by electrical stimulation of touch domes indicated that they are innervated by rapidly conducting A β afferents.

Each touch dome generally is innervated by a single A β afferent that branches repeatedly, ultimately contacting a cluster of ~10–100 epidermal Merkel cells (Fig. 2A; Munger, 1965; Iggo and Muir, 1969). The afferent has large terminals that contact the side of the Merkel cell nearest the basal lamina. This anatomy is reminiscent of other sensory receptor cells, such as inner-ear hair cells. Merkel cells are located in the basal layer of the epidermis, just above the basal lamina. In touch domes, the keratinocytes that overlie Merkel cells are columnar and form more layers, contributing to its dome-like appearance. The dermal portion of the dome contains collagen bundles woven in a tight mesh. The dermis beneath the dome is well vascularized, suggesting high metabolic activity by cells in the touch dome.

Recent experiments by Woodbury and Koerber have confirmed Iggo and Muir's original correlative work indicating that Merkel cell-neurite complexes mediate SAI response (Woodbury and Koerber, 2007). In a technical *tour de force*, they developed a mouse semi-intact preparation to perform electrophysiological recordings from the somata of somatosensory neurons with their cutaneous receptive fields intact. By filling electrodes with a tracer, these authors labeled the afferent projections of recorded neurons. Afferents with SAI-response profiles innervated Merkel-cell clusters in touch domes.

Ultrastructure of Merkel cells

Merkel cell-neurite complexes are found only in highly touch-sensitive areas of skin, such as touch domes, finger tips, whisker follicles and palatine ridges (Fig. 3). Based on this localization and the ultrastructure of Merkel cell-neurite complexes, Merkel cells were first hypothesized to be sensory receptor cells that transduce innocuous touch.

If this model is correct, Merkel cells must fulfill two criteria: 1) they must be intrinsically mechanically sensitive and 2) they must signal the associated afferent through synaptic transmission. Merkel-cell ultrastructure provides some evidence for both functions (Fig. 2B). Merkel cells have thin microvilli, 1–2 μ m in length, which project into overlying keratinocytes (Brown and Iggo, 1967; Iggo and Muir, 1969). These processes are reminiscent of mechanosensitive stereocilia in hair cells and are postulated to detect deformations due to indentation of epithelial surfaces (Iggo and Muir, 1969). Additionally, Merkel cells and keratinocytes are linked by desmosomes, suggesting stiff structural attachments (Munger, 1965; Iggo and Muir, 1969).

Ultrastructural and molecular evidence also supports the notion of synaptic transmission between Merkel cells and the afferent terminal. There is a close association between Merkel cells and enlarged terminals of somatosensory afferents (Iggo and Muir, 1969). The contact between a Merkel cell and an afferent terminal is marked by a synapse-like granular cleft and

a postsynaptic-like thickening of the afferent terminal's membrane (Iggo and Muir, 1969; Hartschuh and Weihe, 1980). In Merkel cells, the presynaptic active-zone protein Piccolo localizes to these contact sites, which confirms that they are presynaptic active zones (Haeberle *et al.*, 2004). Along with Piccolo, Merkel cells express a number of molecules that are essential for evoked synaptic vesicle release, including SNARE-complex proteins, synaptotagmins and voltage-activated Ca^{2+} channels (Haeberle *et al.*, 2004).

Afferent terminals contain an abundance of mitochondria and have small clear-core vesicles (Munger, 1965). In one report, a possible reciprocal synapse was observed with an asymmetrical electron density facing the Merkel cell, accompanied by a cluster of small clear-core vesicles in the neuron (Mihara *et al.*, 1979).

The presence of synapses suggests that Merkel cells are sensory cells that function in the skin much as hair cells and photoreceptors function in hearing and vision; however, Merkel cells do not contain the small clear-core vesicles characteristic of these sensory synapses and fast neurotransmission in general. Instead, Merkel cells contain large, dense-core granules that cluster between their nuclei and the afferent terminal (Mihara *et al.*, 1979; Hartschuh and Weihe, 1980). Large dense-core vesicles are generally thought to release neuropeptides or hormones. Consistent with this notion, immunohistochemical studies demonstrate that Merkel cells produce neuropeptides (Halata *et al.*, 2003), including vasoactive intestinal peptide (Hartschuh *et al.*, 1983) and cholecystikinin octapeptide (Haeberle *et al.*, 2004). Merkel cells also express vesicular monoamine transporter 1, which is an isoform predominantly found in neuroendocrine cells (Weihe *et al.*, 1998).

Dense-core vesicles may also contain the excitatory neurotransmitter glutamate (Morimoto *et al.*, 2003). Thus, Merkel cells may employ large dense-core vesicles for mechanically triggered, excitatory transmission. The recent discovery that Merkel cells express vesicular glutamate transporters further supports the presence of glutamatergic synaptic transmission (Hitchcock *et al.*, 2004; Nunzi *et al.*, 2004). Moreover, whole-cell recordings and live-cell imaging of dissociated Merkel cells revealed voltage-activated ion channels and depolarization-evoked Ca^{2+} transients (Yamashita *et al.*, 1992; Haeberle *et al.*, 2004; Piskowski *et al.*, 2008), indicating that Merkel cells are excitable cells. Although these studies collectively suggest that Merkel cells are presynaptic cells, a direct demonstration that depolarization elicits neurotransmitter release from Merkel cells is still lacking.

Do Merkel cells contribute to mechanotransduction?

These histological and molecular findings have fueled a long-standing controversy over whether Merkel cells, somatosensory afferents or both transduce touch in Merkel cell-neurite complexes. Iggo and Muir (1969) noted that SAI responses have an irregular firing pattern as opposed to the regular firing rate of other slowly-adapting, mammalian mechanoreceptors. This irregular firing pattern could be generated by chemical synaptic transmission between Merkel cells and their afferent terminals, if quanta are released in response to mechanical stimulation with a Poisson-like process (Horch *et al.*, 1974). Tellingly, synaptic transmission has not been implicated in other, regularly firing, cutaneous mechanoreceptors. On the other hand, such firing patterns could be generated by direct mechanical stimulation of the branched afferent terminal itself. Indeed, Gottschaldt and Vahle-Hinz observed SAI mechanoelectric response latencies of ~ 0.2 ms, which they interpreted as being too fast for chemical synaptic transmission (Smith and Creech, 1967; Gottschaldt and Vahle-Hinz, 1981).

Because the location of Merkel cells in the epidermis prevents direct electrophysiological recordings with microelectrodes in intact epithelia, several groups have attempted pharmacological manipulations of semi-intact skin-nerve preparations to determine if synaptic

transmission is required for normal SAI responses. Pacitti and Findlater reported that voltage-activated Ca^{2+} channel inhibitors impair SAI responses in skin-nerve preparations (Pacitti and Findlater, 1988). Additionally, kynurenate, a broad spectrum glutamate-receptor antagonist, reduced the responsiveness of Merkel cell-neurite complexes to touch, indicating that glutamatergic neurotransmission plays a role in touch reception (Fagan and Cahusac, 2001). Furthermore, the peripheral afferents of some somatosensory neurons contain ionotropic glutamate receptors (Coggeshall and Carlton, 1998) and Merkel cells express metabotropic glutamate receptors (Tachibana *et al.*, 2003). The NMDA-type glutamate receptor antagonist MK-801 inhibited the static phase of the SAI response, but an AMPA-type glutamate receptor antagonist had no effect (Cahusac *et al.*, 2005). SAI responses in rat sinus-hair follicles were also reduced by antagonists of two serotonin receptors, 5-HT₂ and 5-HT₃ (He *et al.*, 2003). Surprisingly, these Merkel cell-neurite complexes show immunoreactivity against 5-HT₁ receptors, but not 5-HT₂ and 5-HT₃ (Tachibana *et al.*, 2005). Other antagonists of neuromodulators have been reported to have no effect on SAI responses (Gottschaldt and Vahle-Hinz, 1982).

Studies asking whether Merkel cells are required for the SAI response have produced conflicting results. Eliminating Merkel cells by photoablation greatly diminished SAI responses in one study (Mills and Diamond, 1995) but not in another (Ikeda *et al.*, 1994). The contradictory outcomes can be explained either by incomplete photoablation of Merkel cells or by collateral damage of the somatosensory afferent (Senok *et al.*, 1996). Another study examined slowly adapting responses in mice lacking the low affinity neurotrophin receptor, p75, which have dramatically reduced numbers of touch domes containing Merkel cells (Kinkelin *et al.*, 1999). The authors noted that there were no differences in slowly adapting responses in skin nerve preparations in these mice compared to control animals. Based on these phenotypes the authors conclude that Merkel cells are not required for slowly adapting responses; however, there are a few caveats that confound this interpretation. First, this report did not distinguish between SAI and other slowly adapting fiber types. Second, maximal firing rates of slowly adapting responses were at least an order of magnitude lower ($\sim 5 \text{ s}^{-1}$) than those characteristic of SAI afferents. Finally, touch domes that were detected had only slightly reduced numbers of Merkel cells.

Several groups have attempted to determine whether Merkel cells directly respond to mechanical stimulation. Baumann and colleagues have used a semi-intact sinus hair preparation to investigate Merkel cells *in situ* (Chan *et al.*, 1996). They incubated the entire sinus-hair follicle in fluorescent, Ca^{2+} indicators and treated the preparation with mechanical stimuli and solutions containing depolarizing concentrations of K^+ . Intriguingly, these authors observed that direct touch and hypotonic solutions evoked Ca^{2+} transients in presumptive Merkel cells. Because neuronal contacts are preserved in this preparation, these studies cannot distinguish whether mechanical stimuli directly activate Merkel cells, or whether mechanical stimuli activate afferent terminals, which then signal to Merkel cells through reciprocal synapses (Mihara *et al.*, 1979).

To test the hypothesis that Merkel cells are directly activated by mechanical stimuli, several groups have studied dissociated Merkel cells. Tazaki and Suzuki (1998) observed hypotonic-induced $[\text{Ca}^{2+}]$ increases in a few dissociated Merkel cells labeled with the fluorescent marker quinacrine. Though promising, the difficulty of dissociating and identifying Merkel cells likely prevented analysis of additional cells. To circumvent these challenges, Lumpkin and colleagues have developed methods to dissociate, purify and culture Merkel cells (Haerberle *et al.*, 2004) by employing transgenic mice that specifically express green fluorescent protein (GFP) in Merkel cells in the skin (Lumpkin *et al.*, 2003). Recent studies confirm that Merkel cells are directly activated by hypotonic-evoked cell swelling and indicate that this stimulus is transduced by Ca^{2+} -permeable ion channels (Haerberle *et al.*, 2008). Other groups have failed

to demonstrate mechanically evoked responses in dissociated Merkel cells, which may reflect the loss of Merkel-cell processes in those experimental conditions (Nurse and Cooper, 1988; Yamashita *et al.*, 1992). By contrast, in serum-free culture conditions, Merkel cells display actin-filled processes (Fig. 4) as well as fine, cytoplasmic tendrils (Haeberle *et al.*, 2008).

Merkel cells in the oral epithelium

Merkel cells are abundant in oral epithelia of numerous species, including salamanders, rodents and primates (Tachibana *et al.*, 1997; Halata and Baumann, 1999; Toyoshima *et al.*, 1999). In most species, they are found at highest concentration in palatine ridges (Fig. 3B; Tachibana *et al.*, 1997; Halata *et al.*, 1999; Nunzi *et al.*, 2004). SA responses characteristic of Merkel cell-neurite complexes have been recorded from the goat palate and human oral mucosa (Halata *et al.*, 1999; Trulsson and Johansson, 2002). Nonetheless, several groups have suggested that Merkel cells in the oral cavity may participate in functions other than touch reception. For example, they have been postulated to function in taste-bud morphogenesis in salamanders (Toyoshima *et al.*, 1999) and in wound healing in mammals (Righi *et al.*, 2006).

Morphological evidence suggests that distinct subpopulations of Merkel cells reside in the palate (Tachibana *et al.*, 1997). About half of Merkel cells in the rodent palate are morphologically similar to those in touch domes (Fig. 2 and Tachibana *et al.*, 1997). As described above, these roughly spherical cells are characterized by microvilli (Toyoshima *et al.*, 1998), synaptic contacts with sensory afferents and expression of vesicular glutamate transporters (Nunzi *et al.*, 2004). In the goat palate, only innervated, spheroid Merkel cells have been observed (Halata *et al.*, 1999).

In rodent palates, the remaining Merkel cells, termed dendritic Merkel cells, lack obvious innervation (Tachibana *et al.*, 1997). Instead, they have long cytoplasmic processes containing dense-core vesicles, reminiscent of neuronal projections. Intriguingly, dendritic Merkel cells have recently been described in rat touch domes (Nakafusa *et al.*, 2006). Moreover, Merkel cells dissociated from touch domes adopt this morphology when cultured in the absence of sensory neurons (Fig. 4). Because they apparently lack innervation, dendritic Merkel cells have been proposed to be a functionally distinct subpopulation that releases paracrine signals to influence the development or repair of oral epithelia (Tachibana *et al.*, 1997). Alternatively, dendritic Merkel cells may reflect a developmentally immature state (Kim and Holbrook, 1995). One argument against the latter interpretation is that dendritic Merkel cells are highly abundant in the palates of adult rodents, whereas Merkel cells in the oral cavity develop in the first few postnatal weeks (Tachibana *et al.*, 2000).

Conclusions and open questions

Since their discovery in 1875, Merkel cells have been proposed to function as mechanosensory cells based on their morphology and location (Merkel, 1875; reviewed in Tachibana and Nawa, 2002; Halata *et al.*, 2003). Consistent with this model, recent molecular and physiological studies demonstrate that Merkel cells are neuronal-like cells that are poised to play an active role in touch reception. Despite this progress, key questions remain open. First, is mechanotransduction in Merkel cells necessary for SAI responses? Second, is direct touch sufficient to depolarize Merkel cells and evoke synaptic vesicle release? Third, does neurotransmitter release from Merkel cells send excitatory or modulatory signals to sensory neurons? Alternatively, Merkel cells may release autocrine signals or paracrine signals to surrounding keratinocytes. Finally, do dendritic Merkel cells reflect the development of Merkel cells in adult animals and, if so, what progenitor cell type begets new Merkel cells in mature tissues? This issue is particularly important in the oral epithelium, which is an environment prone to trauma and high mechanical stress (Ishizaki *et al.*, 2006).

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Abbreviations

PC	Pacinian corpuscle
RA	rapidly adapting
SAI	slowly adapting type I
SAII	slowly adapting type II
SNARE	soluble N-ethylmaleimide sensitive factor attachment receptor
5-HT	5-hydroxytryptamine/serotonin

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Figure 1. Representative response profiles of RA, SAI and SAII afferents to touch

Extracellular recordings were made from individual somatosensory afferents using a semi-intact preparation consisting of mouse hairy skin innervated by the saphenous nerve. Plots of instantaneous firing frequency versus time are shown for an RA afferent (conduction velocity = $7 \text{ m}\cdot\text{s}^{-1}$), SAI afferent (conduction velocity = $12 \text{ m}\cdot\text{s}^{-1}$) and SAII afferent (conduction velocity = $11 \text{ m}\cdot\text{s}^{-1}$). Arrows indicate the onset and offset of mechanical stimuli. The prolonged response of the RA fiber reflects vibration in the stimulus probe. Afferents were classified based on conduction velocity, adaptation properties and variance of firing frequency.

Figure 2. Structure of Merkel cell-neurite complexes

(A) A confocal micrograph shows a cluster of Merkel cell-neurite complexes in a mouse touch dome. Skin was dissected from a transgenic *Math1/nGFP* mouse. In these transgenic mice, *Math1* enhancer sequences drive expression of nuclear-localized GFP (green) specifically in Merkel cells in the skin (Lumpkin *et al.*, 2003). Within a touch dome, clusters of Merkel cells are innervated by branches (arrowheads) of a single SAI afferent (red), which was labeled *in vivo* by subcutaneous injection of the styryl dye FM5-95 (Meyers *et al.*, 2003). (B) Schematic of a Merkel cell (green) and its somatosensory afferent (red) in a touch dome (adapted from Iggo and Muir 1969). Merkel cells are connected to overlying keratinocytes by desmosomes and actin-filled microvilli. Large, dense core vesicles are cluster below the Merkel cell's characteristically lobulated nucleus. These vesicles are closely apposed to the afferent terminal and often cluster around synaptic densities. Merkel cells are contacted exclusively by myelinated axons.

Figure 3. Merkel cells (green) cluster in touch domes in hairy skin (A; arrowheads) and palatine ridges in oral epithelia (B; arrows)
Projections of confocal z-series show Merkel cells in intact tissue from *Math1/nGFP* transgenic mice.

Figure 4. Morphology of dissociated Merkel cells

Merkel cells were dissociated from the hairy skin of *Math1/nGFP* transgenic mice, purified by fluorescence-activated cell sorting based on GFP fluorescence (green), then cultured for two days in serum-free keratinocyte media. Actin-filled, dendritic processes were visualized by labeling Merkel cells with rhodamine-phalloidin (red). Details of the isolation and culture methods are given in Haeberle *et al.* (2004 and 2008).