Fine Structure of Eimer's Organ in the Coast Mole (*Scapanus orarius*)

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ABSTRACT

Eimer's organ is a small, densely innervated sensory structure found on the glabrous rhinarium of most talpid moles. This structure consists of an epidermal papilla containing a central circular column of cells associated with intraepidermal free nerve endings, Merkel cell neurite complexes, and lamellated corpuscles. The free nerve endings within the central cell column form a ring invested in the margins of the column, surrounding 1–2 fibers that pass through the center of the column. A group of small-diameter nociceptive free nerve endings that are immunoreactive for substance P surrounds this central ring of larger-diameter free nerve endings. Transmission electron microscopy revealed a high concentration of tonofibrils in the epidermal cells of the central column, suggesting they are more rigid than the surrounding keratinocytes and may play a mechanical role in transducing stimuli to the different receptor terminals. The intraepidermal free nerve endings within the central column begin to degrade 15 μm from the base of the stratum corneum and do not appear to be active within the keratinized outer layer. The peripheral free nerve endings are structurally distinct from their counterparts in the central column and immunocytochemical double labeling with myelin basic protein and substance P indicates these afferents are unmyelinated. Merkel cell-neurite complexes and lamellated corpuscles are similar in morphology to those found in a range of other mammalian skin. Anat Rec, 290:437–448, 2007.

Key words: substance P; Merkel cell; Pacinian corpuscle; mechanosensory; free nerve ending; NF-200; AM1-43; tonofibrils

The coast mole is a small burrowing insectivore that lives in moist soil on the northwestern coast of the United States and Canada. These animals use their sense of touch to navigate and forage for food in dark underground tunnels. They typically tap their nose to the substrate and objects of interest while exploring their environment. A close look at the nose reveals a series of small bumps, or papillae, covering the surface of the glabrous rhinarium. Each papilla is an Eimer's organ (after Eimer, 1871) and nearly every species of the talpid mole is endowed with these structures (Catania, 2000). Microelectrode recordings from somatosensory cortex indicate that Eimer's organ is acutely sensitive to mechanosensory stimuli and have small receptive fields (Catania and Kaas, 1995; Sachdev and Catania, 2002a, 2002b; Marasco and Catania, 2007). Analysis of foraging behavior with high-speed video shows that Eimer's organs are used to make rapid sensory discriminations

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as moles explore their environment (Catania and Kaas, 1997; Catania and Remple, 2004, 2005).

Each Eimer's organ is a complex receptor unit composed of specialized epidermal cells and sensory receptors. A conspicuous feature of Eimer's organ is the central column of epidermal cells extending from the dermis to the skin surface. This central column is invested with 9–27 intraepidermal free nerve endings that enter the base of the organ and extend, in a circular arrangement, directly to the skin surface. Recent immunocytochemical and electrophysiological analyses support the hypothesis that these nerve endings play an important role in the tactile acuity of the organ (Marasco et al., 2006; Marasco and Catania, 2007). A palisade of smaller-diameter peripheral intraepidermal free nerve endings is arrayed around the perimeter of each organ. These outer terminals are positive for substance P, a polypeptide associated with pain transmission, suggesting they play the role in nociception. There are generally 7–15 Merkel cell-neurite complexes at the base of the organ and 1–2 lamellated corpuscles in the dermis below the central cell column.

Eimer's organ is one of the more complex sensory structures to be found in mammalian skin. Our goal in this study was to investigate its fine structure to provide more detailed information about several newly revealed features and to better understand how its morphology might be related to mechanisms of sensory transduction. In particular, we were interested in the differential morphology of the central column free nerve ending receptor terminals as they approached and entered the stratum corneum, as a previous investigation (Marasco et al., 2006) suggested they could extend functional terminals into the outer keratinized layer of epidermis (stratum corneum). We also sought to examine the relationship of the free nerve endings to the epithelial cells and the state of myelination of the newly characterized peripheral free nerve endings. More generally, we examined the structural details of each receptor class found in Eimer's organ in the coast mole for comparison with other species.

MATERIALS AND METHODS

All procedures were approved by the Vanderbilt University Institutional Animal Care and Use Committee and followed the National Institutes of Health guidelines for the care and use of laboratory animals. Five coast moles (*Scapanus orarius*), provided to us by Dr. Kevin L. Campbell of the University of Manitoba (Winnipeg, Canada), were used. The animals were euthanized with a 150 mg/kg intraperitoneal (i.p.) injection of sodium pentobarbital (Euthasol) and perfused through the heart with 0.1 M Na cacodylate buffer followed by 2.5% glutaraldehyde in 0.1 M Na cacodylate buffer. Nasal tissue containing Eimer's organs was removed and postfixed overnight in 2.5% glutaraldehyde in 0.1 M Na cacodylate buffer. The tissue was placed in osmium tetroxide fixative (1%) for 2 hr on ice, dehydrated in a graded ethanol series, and transferred to propylene oxide. The tissue was embedded in Spurr's Resin (EM Sciences, Hatfield, PA) and thin sections were cut at 90–100 nm with a diamond knife on a Leica Ultracut UCT ultramicrotome (Leica, Wetzlar, Germany). The sections were mounted on grids and stained with uranyl acetate (3–3.5%) and lead citrate (2.5%). The sections were viewed on a Phillips CM-12 transmission electron microscope (TEM) at 80 kV.

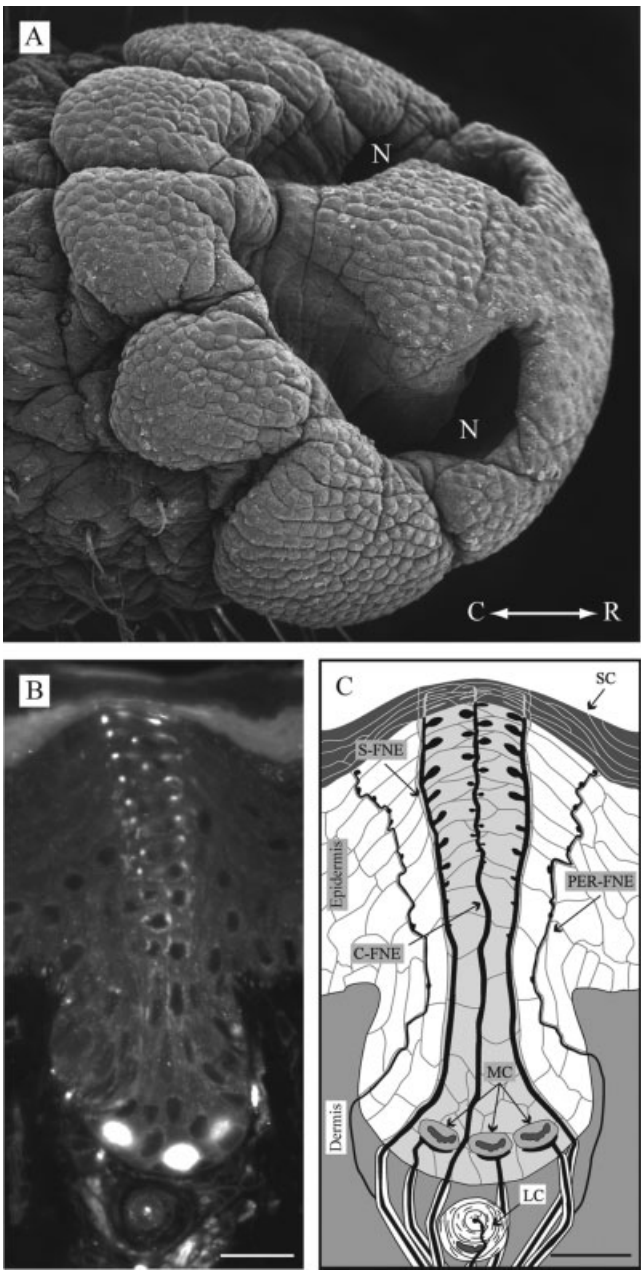

Fig. 1. Eimer's organ of the coast mole (*Scapanus orarius*). **A:** Scanning electron micrograph of the glabrous rhinarium of the coast mole. The small bumps are Eimer's organs. The nose is oriented with the rostral (R) tip to the right and the caudal (C) end to the left. The anterior nares are indicated by N. **B:** Vertical fluorescent confocal micrograph of an Eimer's organ in an animal treated with the neural tracer AM1-43 revealing the terminal swellings of the central column, the Merkel cell-neurite complexes, and the lamellated corpuscle at the base of the organ. **C:** A schematic representation of Eimer's organ showing the organization of the central free nerve ending (C-FNE), satellite free nerve endings (S-FNE), peripheral free nerve endings (PER-FNE), Merkel cell-neurite complexes (MC), and lamellated corpuscle (LC) with respect to the epidermis, dermis, and the stratum corneum (SC). Scale bars = 20 μm.

Animals used for immunocytochemistry were euthanized and the tissue was processed as previously reported (Marasco et al., 2006). Briefly, the moles were

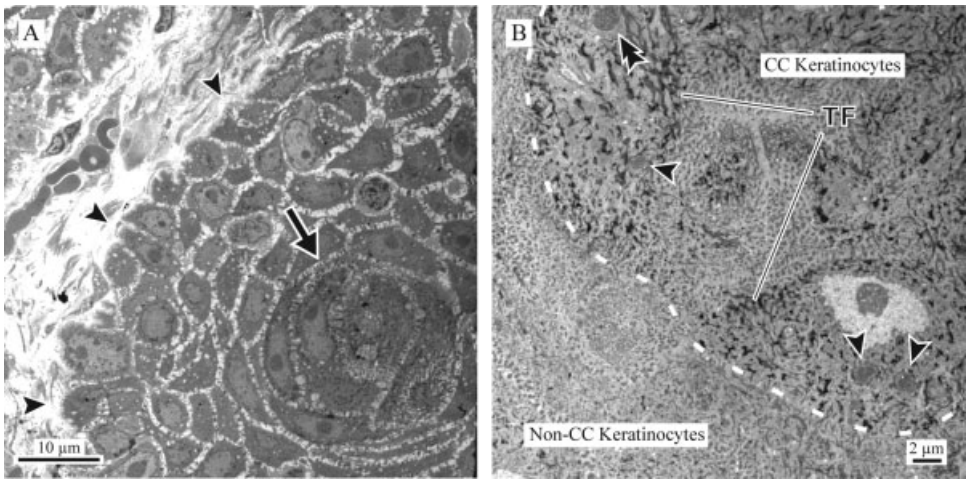

Fig. 2. Two horizontal TEM micrographs of the central column showing the difference in tonofibril concentration between the central column keratinocytes and the surrounding cells. **A:** A view near the base of the organ. The arrow indicates the central column. The cytoplasm of these cells has more electron-dense appearance than the surrounding epithelium. The arrowheads indicate the edge of the epidermal papilla. Micrograph digitally sharpened. **B:** Higher magnification showing the central column keratinocytes with cytoplasmic tonofibrils (TF). The dotted line traces the perimeter of the central column. Three satellite nerve fibers (arrowheads) and one satellite terminal swelling (double arrowhead) are visible.

perfused and the tissue was postfixed in 4% paraformaldehyde, cryoprotected, sectioned on a Jung CM3000 cryostat (Leica) at 20 μm, and mounted on slides. Sections were first blocked and then incubated overnight with goat antimyelin basic protein (anti-MBP) antiserum (Santa Cruz Biotechnology, Santa Cruz, CA) diluted 1:200 and either guinea pig antisubstance P (anti-sP) antiserum diluted 1:20,000 (kindly provided by Dr. John Maggio) or mouse antineurofilament 200 (anti-NF-200; Sigma-Aldrich, St. Louis, MO) diluted 1:800. Following washes, the sections were incubated with an AlexaFluor 488-coupled rabbit antigoat antibody (Molecular Probes/Invitrogen, Carlsbad, CA) and either a Texas Red-coupled donkey antiguinea pig antibody diluted 1:200 (Jackson ImmunoResearch, West Grove, PA) or AlexaFluor 594-coupled donkey antimouse antiserum diluted 1:1,000 (Molecular Probes/Invitrogen). After washing, the sections were coverslipped and imaged on a Zeiss Axioskop microscope running MetaMorph (Molecular Devices, Downingtown, PA).

The animals used for AM1-43 experiments were treated as previously reported (Marasco et al., 2006). In brief, they were injected with AM1-43 (Biotium, Hayward, CA) and then returned to their cages overnight. The dye remained in these animals for an average of 29.5 hr before sacrifice. Following euthanasia and perfusion, the nasal tissue was cryoprotected and sectioned. Images were gathered using a Zeiss Upright LSM510 confocal microscope (Zeiss, Thornwood, NY). All images were processed for brightness and contrast with Photoshop CS2 9.0 (Adobe Systems, San Jose, CA).

RESULTS

Intraepidermal Free Nerve Endings and Associated Epithelium

Each individual Eimer's organ on the surface of the mole's nose is formed from an organized group of epithelial cells (Fig. 1). The most distinct epidermal specialization evident in these organs is the circular column of epithelial cells that run directly through the center of the organ from the basal layer of the stratum spinosum to the skin surface. The central column in the coast mole was generally 2–3 cell diameters wide and these keratinocytes were positioned one above the other in a configuration akin to a stack of coins. When viewed from above, the distinct circular shape of the central column was apparent, surrounded by roughly cuboidal epithelial cells (Figs. 2A and 3A). The keratinocytes of the central column were more electron-dense than the surrounding cells (Fig. 2A). Higher-magnification images revealed networks of tonofibrils within their cytoplasm that were more densely packed than those of the surrounding epithelial cells (Fig. 2B).

The central column of each Eimer's organ was invested for its entire length with intraepidermal free nerve endings. Multiple satellite free nerve endings resided along the outside edge of the column and between one and two central free nerve endings ran directly through the center of the column (Fig. 3A). The outer satellite terminal fibers resided within deep invaginations of the central column keratinocyte plasma membranes. The keratinocytes enveloped these individual terminals, surrounding the fibers such that a junction was often formed between two parts of the same keratinocyte (Fig. 3B). The junction was held tight with interdigitations and desmosomes in the same manner that attachments were made with adjacent keratinocytes (Fig. 3B). In contrast, the central free nerve endings in the center of the column did not sit within a deep cleft in the keratinocyte. Instead, they were generally at the junction in between the two or three epithelial cells that made up the central column (Fig. 3C).

As the central column free nerve endings passed through the epidermis, they formed multiple swellings along their course (Fig. 1). This gave the neurites a bead-on-a-string morphology. The nerve fiber that

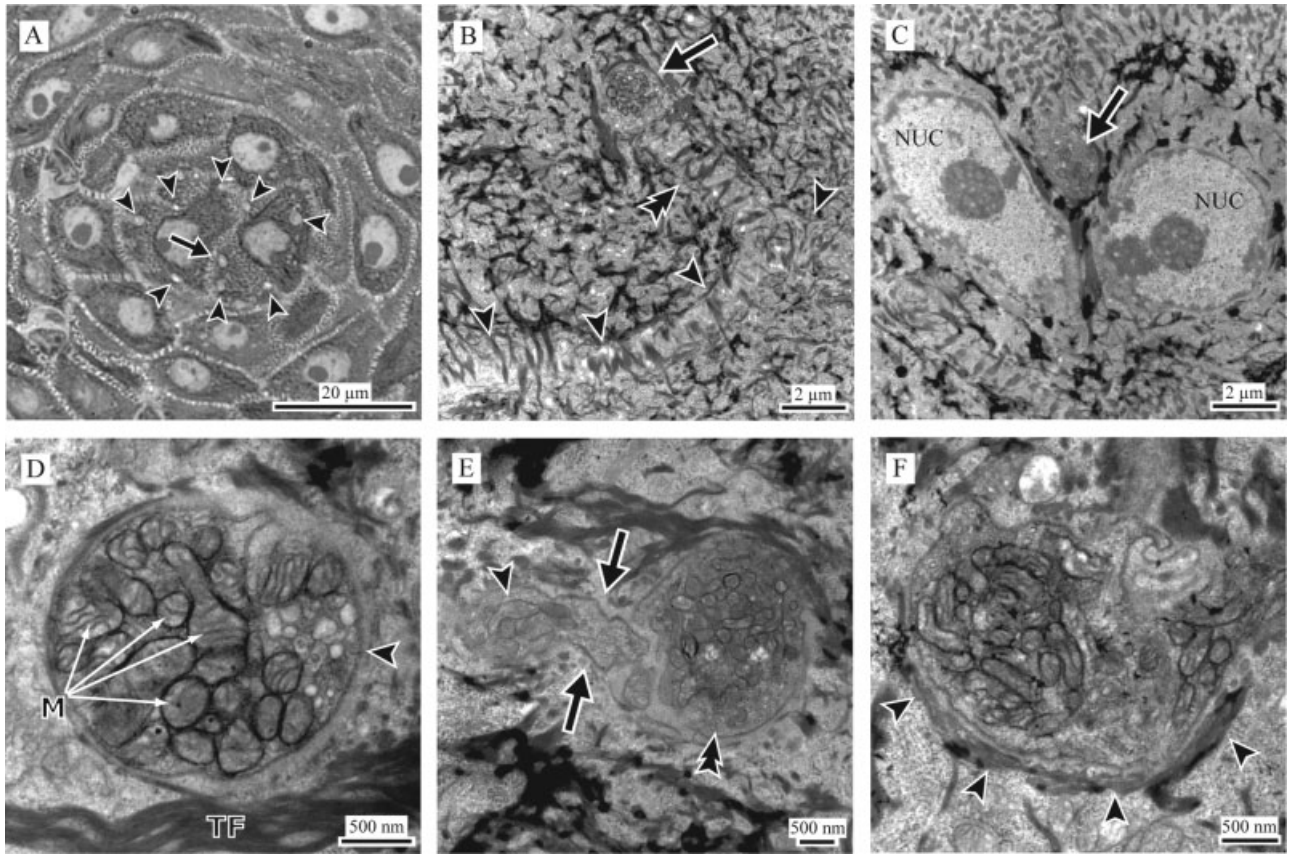

Fig. 3. Multiple views of the central column free nerve ending terminals. **A:** Light micrograph of a toluidine blue-stained section taken parallel to the skin surface near the middle of the epidermis. The arrowheads denote eight satellite fibers around the perimeter of the central column. The arrow points to a single central fiber running through the center of the cell column. **B:** Horizontal TEM micrograph of a single satellite free nerve ending showing the terminal (arrow) enclosed by a central column keratinocyte. The arrowheads point to the junction between the central column keratinocyte and an adjacent epithelial cell. The double arrowhead shows where the plasma membrane of the central column keratinocyte doubles back on itself to enclose the satellite terminal. **C:** Horizontal section of a single center free nerve ending. The center terminal (arrow) is nested between two central column keratinocytes. The nuclei (NUC) of the two cells are in direct apposition to the central fiber terminal. **D:** Horizontal micrograph of a central column fiber terminal swelling. Note the dense accumulation of mitochondria (M) and small zone filled with microvesicles (arrowhead). A group of tonofibrils is evident at the bottom (TF). **E:** A central column free nerve ending terminal swelling. This section passed directly through the neck region (between arrows) that attaches the terminal swelling (double arrowhead) to the neurite (between arrowhead). The neck region is devoid of mitochondria and has a convoluted appearance. **F:** A central column fiber terminal swelling cradled by tonofibrils (arrowheads).

spanned the distance between each terminal was from 0.5 to 1 μm in diameter. The terminal swellings projected toward the center of the column and in thin section they appeared as spheres (Fig. 3D). The cytoplasm of the central column receptor terminals was densely packed with mitochondria and contained numerous microvesicles that were 75–200 nm in diameter (Fig. 3D). The terminals were approximately 3 μm in diameter and they were connected to the nerve fiber by a convoluted neck region that contained many microvesicles but was generally devoid of mitochondria adjacent to the terminal swelling (Fig. 3E). There were also thin networks of supporting tonofibrils apparent at the base of the terminal swellings (Fig. 3F).

The structure of the central column free nerve ending terminals within the stratum spinosum appeared as described above (Fig. 4, B4). As the central column fiber terminal swellings approached the stratum granulosum, they began to degrade at a distance approximately 15 μm below the base of the stratum corneum. At this level, the mitochondria appeared vacuous and the cytoplasm of the terminals became more homogeneous (Fig. 4, B3). This is also the region where neurofilament 200 labeling became less distinct (Fig. 4C). As the neurite terminals entered the stratum corneum, their cytoplasm took on an indistinct, nearly homogeneous, appearance and the outlines of the numerous mitochondria became obscure (Fig. 4, B2). Just deep to the outer skin surface, the terminals had degraded further and there were large voids in their cytoplasm and gaps between the terminal and the surrounding keratinized epidermal cells (Fig. 4, B1).

A second ring of small-diameter intraepidermal free nerve endings formed a palisade around the central column free nerve endings. The peripheral intraepidermal free nerve endings followed a convoluted course to the surface of the organ 1–2 cell diameters removed from the central column epithelial cells (Fig. 5A). In contrast to the circle of central column free nerve endings that resided within the keratino-

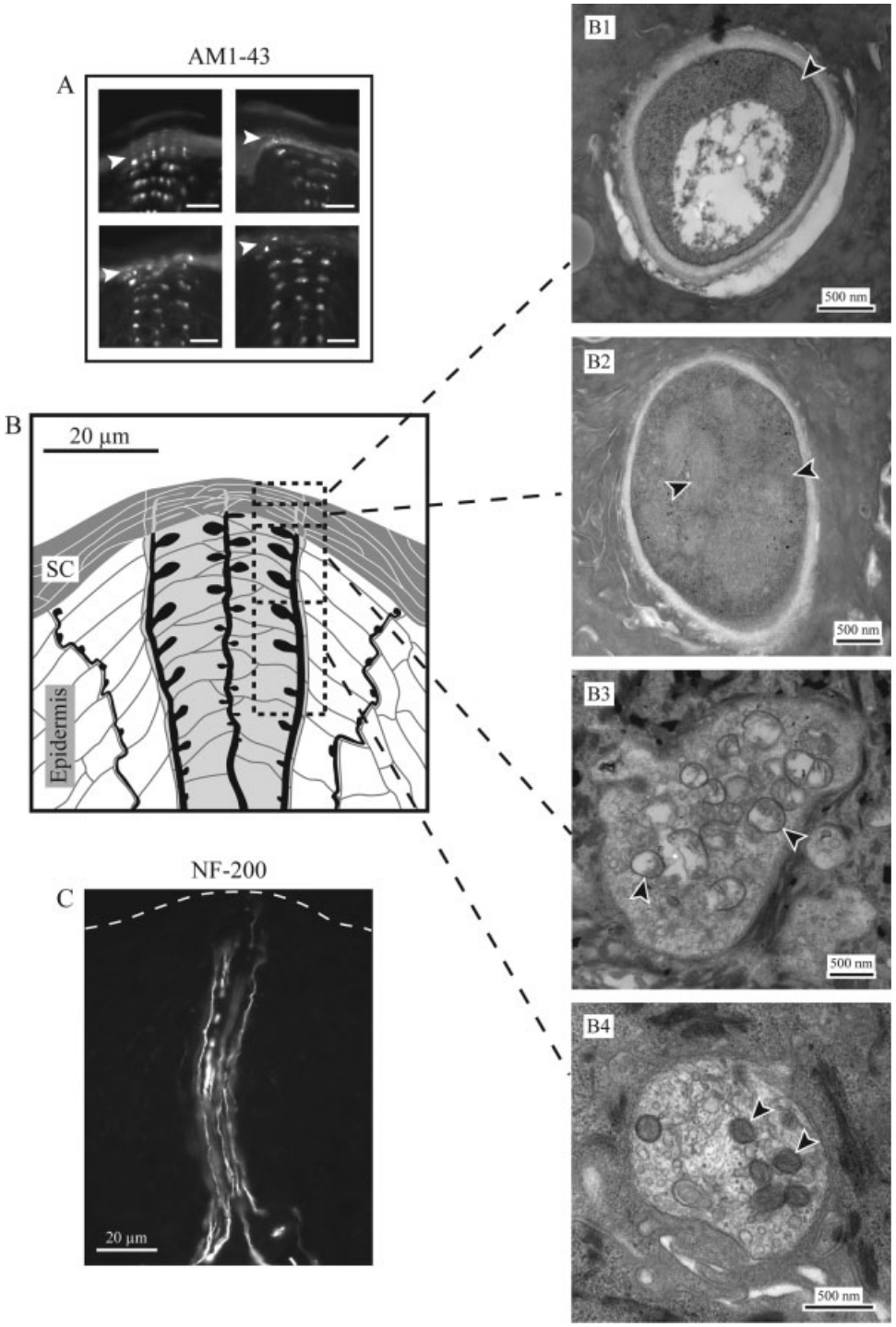

Fig. 4. The condition of the central column free nerve ending terminals as they progress through the epidermis. **A:** Vertical fluorescent confocal micrographs of the apical portions of four different Eimer's organs in animals treated 29 hr previously with AM1-43. Scale bars = 10 μm. The stratum corneum can be seen as a bright horizontal band of autofluorescence. AM1-43-labeled central column fiber terminals can be seen within this band (arrowheads). **B:** A schematic rendering of the tip of an Eimer's organ showing the regions (dotted boxes) where central column terminals at various stages of progression through the epidermis were found (B1–B4, TEM micrographs). B1–B4: Arrowheads indicate mitochondria. B1: A central column free nerve ending terminal at the surface of the skin just before being sloughed off. Note the void within the cytoplasm of the terminal and its disengagement from the surrounding keratinocytes. B2: A central column free nerve ending terminal just after entering the stratum corneum. The mitochondria appear ghostlike and the cytoplasm has a homogeneous appearance. B3: A central column free nerve ending terminal in the initial stages of degradation nearing the stratum granulosum. The cytoplasm has a heterogeneous appearance and contains numerous microvesicles. However, the mitochondria are degrading and appear vacuous. B4: A central column free nerve ending terminal within the stratum spinosum. The mitochondria appear intact and the cytoplasm is heterogeneous with many microvesicles. **C:** Vertical fluorescent light micrograph of an Eimer's organ immunolabeled for NF-200. The dotted white line represents the location of the base of the stratum corneum. Immunoreactivity diminishes 15–20 μm below the base of the stratum corneum.

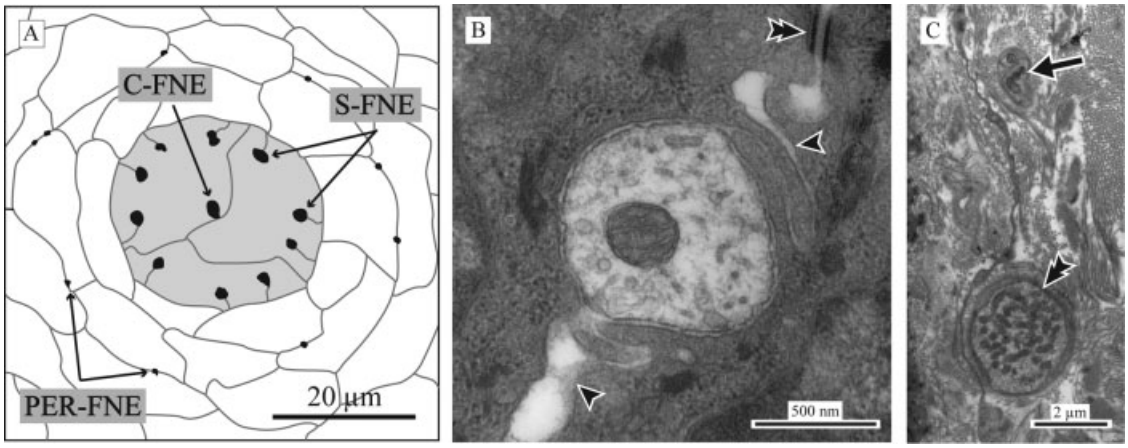

Fig. 5. The peripheral intraepidermal free nerve endings. **A:** Schematic representing the central column (shaded grey) and the relationship of the peripheral nerve endings (PER-FNE) to the satellite nerve endings (S-FNE) and the central nerve ending (C-FNE). The peripheral free nerve endings form a palisade around the central column (filled in gray) and central column free nerve endings at a distance of 1–2 cell diameters from the central column. **B:** A peripheral free nerve ending nested within the junction (arrowheads) between two different keratinocytes. The cytoplasm of these terminals is sparse in mitochondria and microvesicles. Double arrowhead points to a desmosomal attachment between adjacent epithelial cells. **C:** This micrograph within the dermal compartment illustrates the size difference between the peripheral free nerve endings (arrow) and the central column free nerve endings (double arrowhead). There are two peripheral free nerve ending fibers within the bundle marked by the arrow.

cytes at the margins of the cell column, the peripheral free nerve endings tracked within the interstitial space between adjacent epithelial cells. The peripheral free nerve endings also showed swellings intermittently along their course but these were generally smaller than 1 μm in diameter. Much of the cytoplasm of the peripheral free nerve endings was sparsely filled with mitochondria and microvesicles (Fig. 5B). However, occasionally peripheral free nerve endings were seen to be densely packed with mitochondria. Figure 5C illustrates the distinct size disparity between the peripheral and central column free nerve endings at the level of the dermis.

As the peripheral free nerve endings exited the epidermis and entered the dermis, they immediately converged into small bundles (Fig. 6A). Further down in the dermis, the smaller peripheral free nerve ending bundles converged into larger bundles (Fig. 6B). These larger bundles then joined with the large sensory nerve fascicles that serve each Eimer's organ. At this point, the mechanosensory afferents that serve the organs were fully myelinated. However, the peripheral free nerve ending bundles within the larger nerves appeared to be unmyelinated (Fig. 6C). Staining of the deep nerve fiber bundles that serve the organs with anti-sP and anti-MBP showed that the sP-positive fibers resided outside and in between the small rings of MBP immunoreactivity (Fig. 6D–G). Substance P is a marker for nociceptive primary afferent neurons (Kuraishi et al., 1985; Lawson et al., 1997) and MBP is a marker for the peripheral myelin sheath (Mendell and Whitaker, 1978; Kimura et al., 1989). When the nerve fibers were labeled for MBP and NF-200, a different pattern emerged. The labeling for NF-200, a marker for large-diameter myelinated peripheral mechanoreceptors (Lawson and Waddell, 1991; Sann et al., 1995), was confined within the circles of MBP immunoreactivity (Fig. 6H–J).

Merkel Cell-Neurite Complexes

There was a collection of Merkel cell-neurite complexes lying in a flat circular arrangement roughly parallel to the skin surface in the stratum germinativum at the base of each Eimer's organ (Fig. 7A). Each Merkel cell was slightly elliptical with the long axis in parallel to the skin surface and was approximately 10–12 μm at its widest. The deep portion of the cell resided within a cup formed by the closely associated terminal neurite disk. The terminal neurite itself was filled with mitochondria and numerous small clear vesicles. The interface between the Merkel cell and surrounding epithelial cells was characterized by many small interdigitations and desmosomal connections. The junctions at the lateral aspects of the cell tended to be larger than those found on the superficial portion. In addition, there were multiple rod-like extensions of the Merkel cell cytoplasm that penetrated deep into cytoplasmic invaginations in the adjoining epithelial cells. The processes themselves were devoid of desmosomes; however, desmosomes were present at the base of each extension. The nucleus of the Merkel cell was multilobulated and occupied a considerable portion of the center of the cell. Golgi complexes were positioned in the cytoplasm superficial to the nucleus. There were numerous mitochondria in the cytoplasm and they tended to be mostly concentrated around the nucleus outside of the region between the nucleus and the neurite disk (Fig. 7B). The cytoplasm between the nucleus and terminal neurite contained multiple membranous dense-cored granules (Fig. 7C).

Lamellated Corpuscles

There were between one and two lamellated corpuscles located in the dermis directly beneath the epidermal central column cells of each Eimer's organ. These corpuscles were approximately 17.5 μm in diameter and 45 μm long. Each lamellated corpuscle was innervated by a single terminal neurite aligned parallel to the surface of the skin. The terminal neurite was elliptical in cross-section, approximately 3.5 μm across the

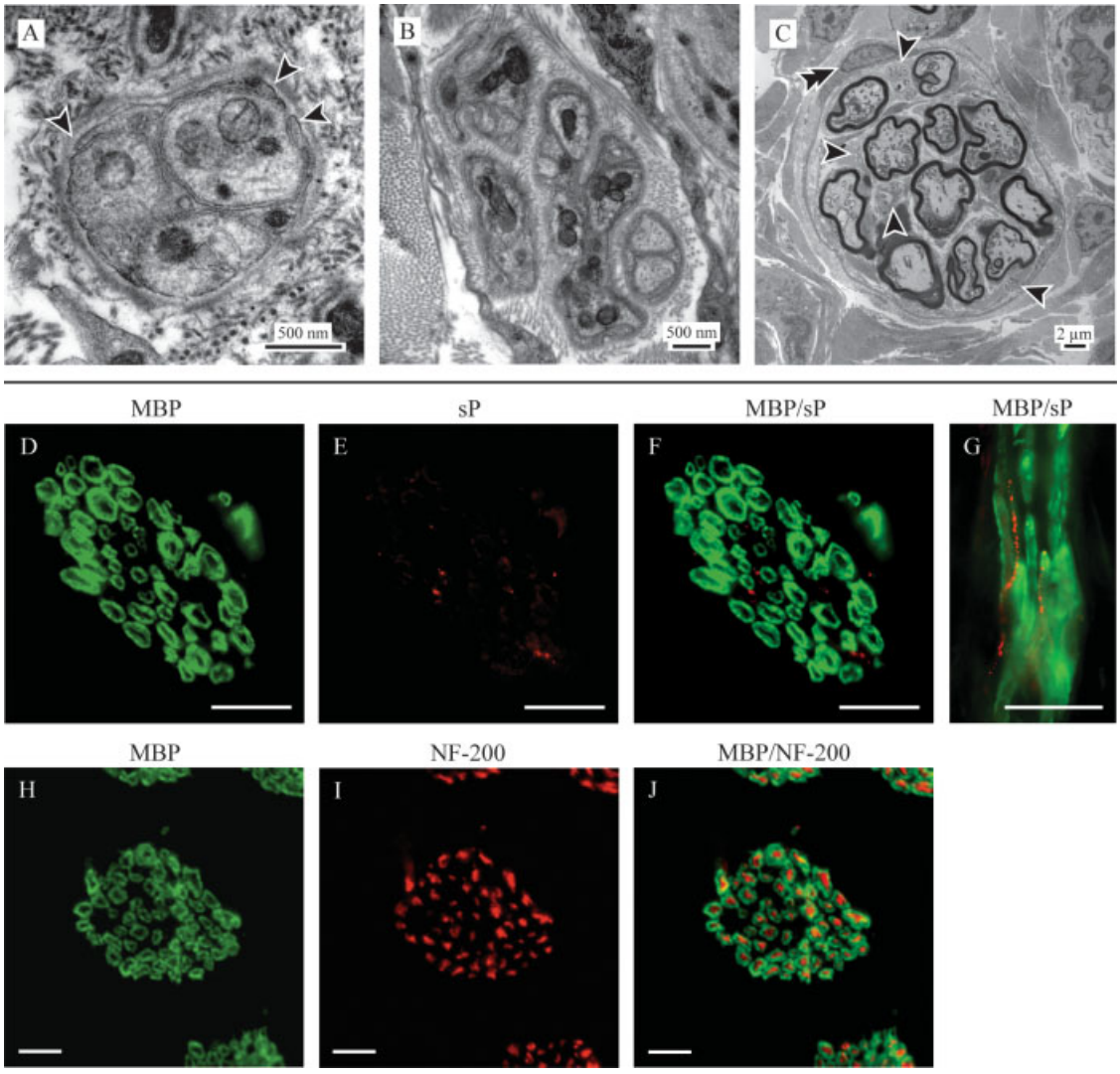

Fig. 6. The cellular sheath of the peripheral free nerve endings compared to the sheaths of the mechanosensitive elements of Eimer's organ. **A:** A small fiber bundle of peripheral free nerve endings within the dermis at the level where the individual neurites have just left the epidermis. The bundle is enclosed within the processes of a single Schwann cell. Arrowheads point to the enveloping Schwann cell. **B:** A representative peripheral free nerve ending bundle deeper in the dermis where multiple fibers have converged. **C:** A representative nerve fascicle serving an Eimer's organ. The locations of four peripheral free nerve ending bundles are marked by arrowheads. The nucleus of the perineural cell ensheathing the fascicle can be seen (double arrowhead). **D–J** are fluorescent light micrographs of nerve fascicles serving Eimer's organs, which are immunoreactive for MBP, sP, and NF-200. Scale bars = 20 μm. D: Immunoreactivity for MBP showing the myelin sheaths of afferent neurons. E: Substance P. The marker for the peripheral free nerve endings is seen as small puncta within the nerve fascicle. F: The overlay illustrates that sP^+ nerve fibers are unmyelinated. G: A longitudinal overlay of two sP^+ fibers in a separate nerve fascicle. H: MBP shows myelin sheaths of the afferent neurons. I: NF-200 distributed throughout the nerve fascicle. J: In this overlay, NF-200 is present in the center of each MBP^+ ring.

widest axis, and contained multiple mitochondria and small vesicles. Two distinct concentric zones of lamellations surrounded the neurite. There was a 4 μm thick, layered, electron-lucent zone that surrounded the terminal neurite and a 3–4 μm thick zone of circularly layered cells that had a grainy, electron-dense, cytoplasm surrounding the internal region (Fig. 8A). Within the lighter inner region, multiple layers of bilaterally symmetrical hemilamellae were seen in close apposition to the neurite. These hemilamellae extended approximately 2 μm out from the terminal neurite. Occasionally, an extension of the terminal neurite extended perpendicularly from the central axis into the cleft between the hemilamellae (Fig. 8B and C). This structure was similar to the filopodia described by Bolanowski et al. (1994) and Kruger et al. (2003b). In addition, there was a 2 μm thick band of more concentrically organized lamellations surrounding the hemilamellae. These two structures within the lighter zone appeared to correlate to the inner and outer cores that are generally seen in larger Pacinian corpuscles (Pease and Quilliam, 1957; Bolanowski et al., 1994). Three concentric layers of cells with

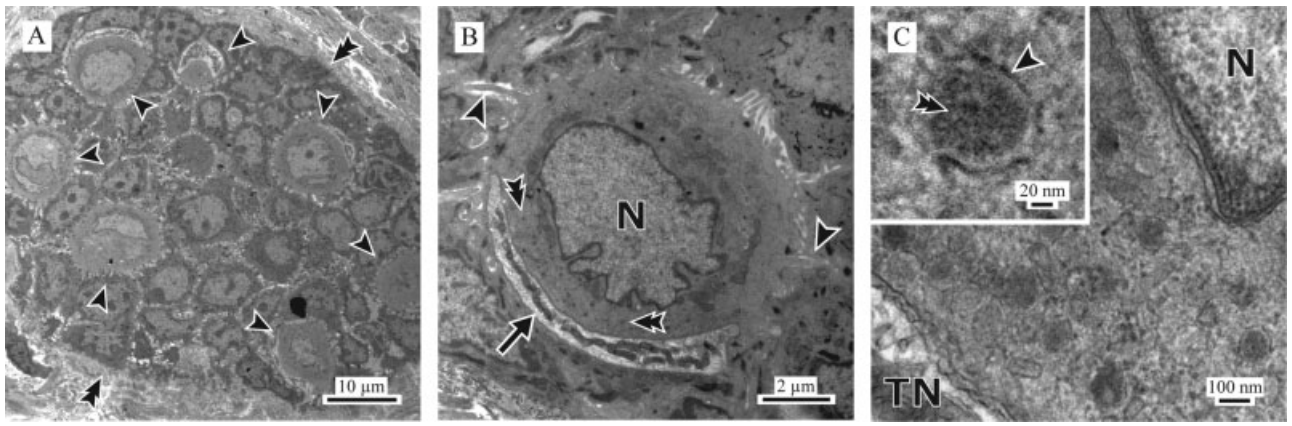

Fig. 7. Merkel cell-neurite complexes. **A:** A horizontal TEM micrograph of the base of an Eimer's organ showing Merkel cells (arrowheads) and edges of the epidermal papilla (double arrowheads). Six Merkel cell-neurite complexes are visible (arrowheads). **B:** A single Merkel cell-neurite complex showing the lobulated nucleus (N) and adjoining terminal neurite disk (arrow). Rod-like extensions of the Merkel cell extend into adjacent keratinocytes (arrowheads). A number of dense-cored vesicles are visible between the nucleus and neurite disk (double arrowheads). **C:** The region between the terminal neurite disk (TN) and the nucleus (N). The dense-core vesicles can be seen as dark inclusions. Inset: This shows a single vesicle. A circular electron-dense (double arrowhead) region surrounded by a membranous envelope (arrowhead).

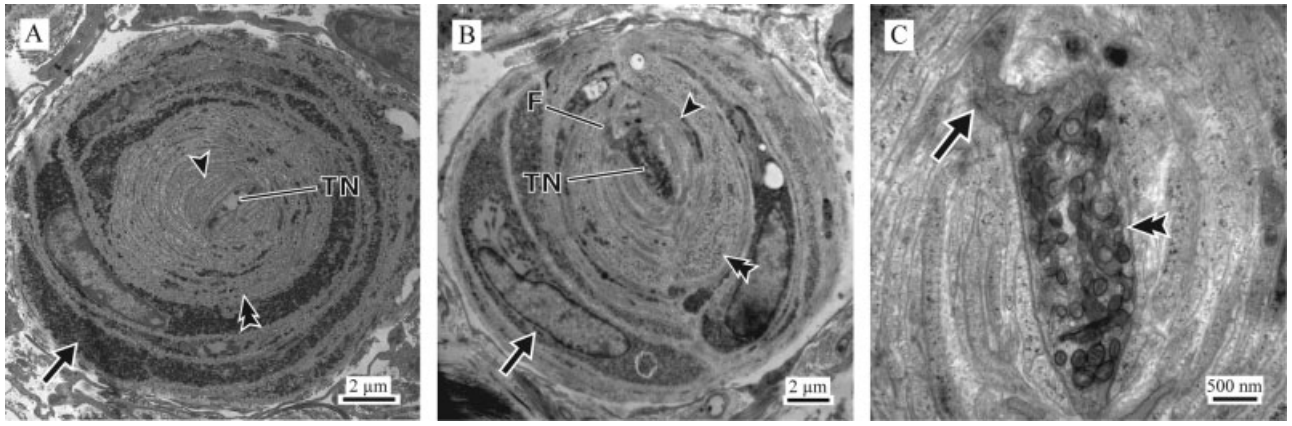

Fig. 8. Lamellated corpuscles. **A:** A single lamellated corpuscle in cross-section. The terminal neurite (TN) is enclosed within the hemilamellations of the internal core (arrowhead), followed by the circular lamellations of the outer core (double arrowhead). The terminal neurite, inner core, and outer core are enclosed by the outer capsule (arrow). **B:** A lamellated corpuscle in cross-section. The inner core (arrowhead), outer core (double arrowhead), and outer capsule (arrow) are evident. However, the entire capsule surrounding the terminal neurite appears as two ellipsoid caps. A filopodial structure extending into the cleft between the hemilamellations is evident (F). **C:** Close-up of the neurite in B. The neurite is packed with mitochondria and microvesicles (double arrowhead). The filopodial structure (arrow) extends into the cleft of the hemilamellations and is devoid of mitochondria.

electron-dense cytoplasm formed the outer region. These lamellations were much less tightly wrapped than those within the inner portion of the corpuscle and the nuclei of the cells were clearly visible. This outer region appears similar to the outer perineural capsule of the Pacinian corpuscle (Andres and von During, 1973; Molinovsky et al., 1990; Andres et al., 1991).

DISCUSSION

The Eimer's organ of the mole is an interesting mechanosensory complex that is formed from both epidermal and neural specializations. Most other mole species share this particular adaptation, and it appears to play a major role in tactile acuity (Catania, 2000). A larger structure in the monotremes called a push rod has a similar configuration of epithelial cells and sensory receptors (Andres and von During, 1984; Andres et al., 1991; Manger and Hughes, 1992; Iggo et al., 1996; Manger and Pettigrew, 1996). In star-nosed moles, Eimer's organ is smaller, with a central cell column only one cell in diameter and free nerve endings more closely associated with one another (for details, see Catania, 1996). The mole, platypus, and echidna all rely on touch to an important extent as they forage for small prey in environments where there is little visual information. Moles and monotremes appear to have developed a convergent structure, using common components of mammalian skin, to maximize tactile sensitivity.

Tonofibrils and Central Column

Tonofibrils serve as a structural support network that imparts rigidity to the cell and provides resilience to mechanical stresses (Janmey et al., 1991; Goldman et al., 1996; Yoon et al., 2001). The central column cells of the Eimer's organ are heavily invested with tonofibril bundles (Figs. 1 and 2). A similar arrangement of tonofibrils is seen in the push rod and it has been suggested that the central column cells function as a rigid rod (hence the name), which is supported by more flexible cells that can be bent from tangentially applied stimuli or moved up and down with compressive stimuli (Andres and von During, 1984; Manger and Pettigrew, 1996). It is interesting to note that the 19th-century anatomist who first described the push rod likened it to the "push" (push-button) of a doorbell and suggested that it served to transmit surface pressure to the terminals at the base of the organs (Poulton, 1885).

It has been proposed that Eimer's organ of the mole may function to bend and conform to objects possibly relating fine textural details (Catania, 2000). The position of the central column at the center of the organ and extending to the surface of the epithelial dome may provide a means for the entire structure to respond to very slight indentations, thus allowing for a high degree of tactile acuity. The central column free nerve endings in particular, located in a circular arrangement along the outside edge of the central column, are in an ideal position to register the mechanical stresses to the deflection of the column in different directions (Catania, 2000). This directional sensitivity has also been suggested for the vesicle chain receptors of the push rod (Andres and von During, 1984; Andres et al., 1991). We have recently been recording from the trigeminal afferents supplying Eimer's organ in the coast mole and found strong support for this suggested function (Marasco and Catania, 2007). Specifically, many of the afferents were selectively activated by sweeping stimulation to the Eimer's organs in one favorable direction. We also found exceptionally small receptive fields for arrays of Eimer's organs. This is consistent with their morphology, as the punctate nature of the organs and cell column presumably restricts mechanical deflections from being transmitted to wide regions of the skin surface, thus increasing tactile acuity (Manger and Pettigrew, 1996; Marasco et al., 2006; Marasco and Catania, 2007).

Condition of Central Column Free Nerve Endings Within Stratum Corneum

Studies by Gale et al. (2001) and Meyers et al. (2003) provide evidence that the sensory neural tracer AM1-43, a styryl pyridinium dye, enters mechanosensory cells in an activity-dependant manner. Recently, this dye was used to label the elements of Eimer's organ in the coast mole (Marasco et al., 2006). As would be predicted from their proposed mechanosensory function, the terminal swellings of neurites in the central column were heavily labeled after approximately 30 hr. However, it was of great interest to see dye apparent in nerve terminals within the outer layer of stratum corneum. This result suggested exceptionally superficial nerve terminals might be transducing sensory information in Eimer's organ. This would be surprising, as we are unaware of active sensory terminals in this skin layer in other species, and because ultrastructural evidence from the star-nosed mole indicates that the central column free nerve ending terminals are degraded and nonfunctional in the stratum corneum (Catania, 1996). In this study, we focused considerable attention on the state of the central column free nerve ending terminals within the stratum corneum of the coast mole to determine if they might be functional from a morphological perspective.

We found that the central column terminals of the coast mole began the process of degradation in the living epidermis approximately 15 μm from the stratum corneum (Fig. 4B). It is interesting to note that immunoreactivity for NF-200 also drops off considerably as the terminals approach the stratum corneum (Fig. 4C). This zone where the reduction in NF-200 immunoreactivity occurs appears to coincide with the region where the central column free nerve ending terminals begin to undergo ultrastructural degradation. Together, these findings suggest the terminals are not functional as they progress into the statum corneum and appear to degrade at a position even deeper in the epidermis than those of the star-nosed mole. This raises the question of why the terminals should be labeled by AM-143?

The stratum corneum directly over the central column in the coast mole is approximately eight cell layers thick. In mouse, the stratum corneum of the footpad is 31 cell layers thick and is entirely turned over within 198 hr (Kvidera and Mackenzie, 1994). Since the mole uses its nose to probe relatively abrasive soil, it would be reasonable to assume the rate of turnover for the cells in the nose is similar, if not faster, considering the high metabolic rate of insectivores. Using the turnover rate for the mouse footpad stratum corneum as a guide, the hypothetical turnover rate for the mole's nose stratum corneum could easily be approximately 50 hr. After 30 hr, cells entering the stratum corneum would progress over halfway through. This equates well to what is seen in Figure 4A, where AM1-43 is found progressing through the stratum corneum after being in the system for 29 hr. It appears that the presence of AM1-43 in the terminals within the stratum corneum is most likely due to the retention of label in terminals that were active in the nonkeratinized epidermis and were subsequently transported to this layer in the normal progression of the epidermal cell layers. Given the morphology of the nerve terminals within the stratum corneum (Fig. 4), it seems very unlikely the presence of AM-143 is a reflection of receptor activity at this level in the skin.

Structure of Peripheral Free Nerve Endings and Nature of Their Cellular Sheath

Early anatomists initially described smaller fiber terminals residing outside of the central column in the European mole (Eimer, 1871; Ranvier, 1880), but they were seldom identified in subsequent studies. Their significance and consistency across species remained obscure until a recent investigation with an immunocytochemical marker directed toward substance P (Marasco et al., 2006). This study routinely revealed a palisade of small free nerve endings surrounding the central column free nerve endings that was positive for substance P (Marasco et al., 2006).

In an effort to learn more about the peripheral free nerve endings, we examined their general morphology, their relationship to the keratinocytes, and their course within the dermis. In our initial confocal study, these fibers appeared to follow a convoluted course between the keratinocytes (Marasco et al., 2006). Under TEM, the fibers were found within the interstitial space between adjoining keratinocytes, confirming that the fibers coursed between epithelial cells to reach the skin surface instead of being enveloped by the keratinocyte like the central column free nerve endings. The peripheral terminals are also much smaller than their central column free nerve ending counterparts and tend to have smaller swellings along their length. In addition, the peripheral neurites contain far fewer mitochondria and microvesicles than the central column fibers (Fig. 5B and C). It should be noted that the morphological qualities of both the central column free nerve endings and peripheral free nerve endings correspond well to those reported for the varicose (beaded) and fine (plain) free nerve endings, respectively, in human digital skin (Cauna, 1980). However, the peripheral terminals in this system appear to lack a distinct axonal reticulum and dense aggregations of microvesicles and appear to differ somewhat from the canine and rodent testicular free nerve endings described by Kruger et al. (2003a), though without serial sectioning the length of the terminals, the presence of these structures cannot be ruled out.

In an effort to gain an understanding of the nature of the cellular sheath surrounding the peripheral free nerve endings, we used semiserial sectioning (four thin sections every 1 μm) to follow the courses of these fibers deep into the dermal compartment. As the peripheral neurites left the epidermis, they immediately coalesced into small bundles with 2–3 separate fibers (Fig. 6A). The bundles were wrapped with a single Schwann cell, and as they coursed deeper into the dermis, more small fibers joined together to form larger bundles with no evidence for a myelin sheath (Fig. 6B). Deep within the dermis, the peripheral free nerve ending bundles converge with the large nerves that serve the individual Eimer's organs. The large afferents within the nerves were myelinated; however, the peripheral neurite bundles, composed of between three and eight fibers, remained unmyelinated and resided in the spaces between the myelinated afferents (Fig. 6C). In previous examinations with immunocytochemical markers, the peripheral free nerve endings were found to be immunoreactive for sP and the central column free nerve endings, lamellated corpuscles, and Merkel cell-neurite complexes were immunoreactive for NF-200 (Marasco et al., 2006). We used this distinction to verify the location and state of myelination of the peripheral free nerve endings within the afferent nerve fascicles that serve the organs. Immunolabeling for sP and NF-200 was compared to immunolabeling for MBP. Within the large afferent nerves, NF-200 immunoreactivity was restricted within the circles of MBP (Fig. 6H–J). Conversely, sP immunoreactivity was only apparent outside of the rings of MBP labeling (Fig. 6D–G). The position of the sP labeling in relationship to the MBP and NF-200 labeling correlates well to the position of the peripheral free nerve ending bundles seen with TEM. These results suggest that the sP-positive components of the peripheral terminal ring are derived from small-diameter unmyelinated nociceptors.

Structure of Merkel Cell-Neurite Complexes

The structure of the Merkel cell-neurite complexes in the coast mole appears very similar to that typically reported for Merkel cell-neurite complexes in many different species (Munger, 1965; Iggo and Muir, 1969; Andres and von During, 1973; Gottschaldt and Vahle-Hinz, 1981; Munger and Ide, 1988). In particular, the Merkel cell-neurite complexes in the coast mole have prominent rod-like cytoplasmic extensions into surrounding keratinocytes and deeply lobulated nuclei. In addition, the Merkel cell-neurite complexes also have numerous dense-cored vesicles concentrated between the nucleus and the adjacent terminal neurite disk (Fig. 7B and C). Given this, it is reasonable to suggest that they provide a slowly adapting SA-1 signaling component to the Eimer's organ receptor complex (Iggo and Muir, 1969; Gottschaldt and Vahle-Hinz, 1981). The circular arrangement of these receptors around the base of the central column, in conjunction with the presumably more rigid cells of the central column, may also provide these receptors with the ability to code for directional input as provided by differential pressure transmitted through the central column (Quilliam, 1966).

Structure of Lamellated Corpuscles

The Pacinian corpuscle in the mesentery of the cat measures 1,000 μm by 670 μm (Quilliam and Sato, 1955). The lamellated corpuscles in the coast mole measure approximately 45 μm by 17 μm. Aside from the substantial difference in size between the typical Pacinian corpuscle in the cat and the lamellated corpuscle in the coast mole, these two receptor end organs appear to share a number of similarities. In one section in particular, a set of eight bilaterally symmetrical hemilamellations was evident surrounding the elliptical terminal neurite (Fig. 8A). This 2 μm thick inner core was surrounded by a 1.9 μm thick ring of 10 circular lamellations, most likely representative of the outer core (Pease and Quilliam, 1957; Bolanowski et al., 1994). The inner and outer cores were surrounded by a large 3 μm thick outer capsule consisting of four circular lamellations. However, this outer capsule was somewhat different from the typical Pacinian capsule as it is quite electron-dense and is composed of more lamellations than is generally reported (Andres and von During, 1973; Molinovsky et al., 1990; Andres et al., 1991). In addition there was evidence of filopodial structures extending from the terminal neurite into the cleft between the hemilamellations (Bolanowski et al., 1994; Kruger et al., 2003b). Thus, despite being smaller than the typical Pacinian corpuscle, the lamellated corpuscle of the coast mole has many of the structural components in place that suggest it is a rapidly adapting FA-2 sensory receptor (Loewenstein, 1958, 1961; Sato, 1961).

The results of this investigation provide new details regarding the structure and function of Eimer's organ in moles. They are consistent with recent investigations of electrophysiological (Marasco and Catania, 2007) and immunocytochemistry (Marasco et al., 2006), suggesting there are at least four different sensory components in association with each Eimer's organ. These include lamellated corpuscles in the dermis, Merkel cell-neurite complexes in the epidermis, and two different classes of

free nerve endings in the epidermis. Lamellated corpuscles and Merkel cell-neurite complexes have been well characterized physiologically in other species and are generally considered rapidly and slowly adapting mechanoreceptors, respectively. The peripheral free nerve endings that form a ring around the perimeter of each Eimer's organ contain substance P, suggesting they act as nociceptors. This role is also consistent with their position and the delicate nature of the skin surface. The nerve fibers and terminal swellings in the central cell column are hypothesized to act as mechanoreceptors that transduce direction information regarding the deflection of the cell column during each touch. New support for this hypothesis comes from a recent study of trigeminal afferent responses (Marasco and Catania, 2007).

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