Elaboration and Innervation of the Vibrissal System in the Rock Hyrax (Procavia capensis)

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FSCs. The presence of vibrissae covering the hyrax body presumably facilitates navigation in the dark caves and rocky crevices of the hyrax’s environment where visual cues are limited, and may alert the animal to predatory or conspecific threats approaching the body. Furthermore, the presence of vibrissae on the postfacial body in both manatees and hyraxes indicates that this distribution may represent the ancestral condition for the supraorder Paenungulata.

Key Words
Vibrissae · Follicle-sinus complex · Somatosensory · Merkel endings · Evolution · Immunohistochemistry

Abstract
Mammalian tactile hairs are commonly found on specific, restricted regions of the body, but Florida manatees represent a unique exception, exhibiting follicle-sinus complexes (FSCs, also known as vibrissae or tactile hairs) on their entire body. The orders Sirenia (including manatees and dugongs) and Hyracoidea (hyraxes) are thought to have diverged approximately 60 million years ago, yet hyraxes are among the closest relatives to sirenians. We investigated the possibility that hyraxes, like manatees, are tactile specialists with vibrissae that cover the entire postfacial body. Previous studies suggested that rock hyraxes possess postfacial vibrissae in addition to pelage hair, but this observation was not verified through histological examination. Using a detailed immunohistochemical analysis, we characterized the gross morphology, innervation and mechanoreceptors present in FSCs sampled from facial and postfacial vibrissae body regions to determine that the long postfacial hairs on the hyrax body are in fact true vibrissae. The types and relative densities of mechanoreceptors associated with each FSC also appeared to be relatively consistent between facial and postfacial

Abbreviations used in this paper

| Abbreviation | Description                           |
|--------------|---------------------------------------|
| BSA          | bovine serum albumin                  |
| CGRP         | calcitonin gene-related peptide       |
| CS           | cavernous sinus                       |
| DVN          | deep vibrissal nerve                  |
| FNE          | free nerve ending                     |
| FSC          | follicle-sinus complex (vibrissa)     |
| ICB          | inner conical body                    |
| MBP          | myelin basic protein                  |
| MECs         | Merkel-ending complexes               |
| NF200        | 200-kDa subunit of neurofilament      |
| NPY          | neuropeptide Y                        |
| OCB          | outer conical body                    |
| PBS          | phosphate-buffered saline             |
| PGP          | protein gene product 9.5              |
| RRC          | rete ridge collar                     |

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Introduction

Together with Proboscidea (elephants) and Sirenia (manatees and dugongs), the Hyracoidea (hyraxes) form the supraorder Paenungulata, or ‘nearly ungulates’ [Simpson, 1945; Ozawa et al., 1997]. Extant hyraxes comprise four species in three genera within the family Procaviidae, two tree hyraxes (southern tree hyrax, *Dendrohyrax arboreus*; western tree hyrax, *D. dorsalis*) and two rock hyraxes (rock hyrax, *Procavia capensis*; yellow-spotted rock hyrax, *Heterohyrax brucei*) [Shoshani, 2005]. Rock hyraxes are diurnal, rabbit-sized herbivores that inhabit rocky outcrops and mountainous regions predominantly in sub-Saharan Africa [Crandall, 1964; Walker et al., 1964; Stoddart and Fairall, 1981; Olds and Shoshani, 1982]. They have a life span of approximately 12 years, subsist mainly on grasses, a low-quality and often scarce food source [Hoeck, 1975; Olds and Shoshani, 1982], and also exhibit poor thermoregulation, forcing them to huddle together in low temperatures in order to conserve body heat [Sale, 1970b] and to restrict feeding activities to cooler periods of each day [Hoeck, 1975; Brown and Downs, 2006]. Hyraxes generally live in family groups of one adult territorial male, several adult females and their young [Hoeck, 1975], and though they feed and sunbask as a group, there is a high level of intraspecific aggression [Fourie, 1977].

The orders Hyracoidea and Sirenia are thought to have diverged nearly 60 million years ago [Springer et al., 2003; Pardini et al., 2007], although the phylogeny of Afrotheria remains intensely debated based on analyses that focus on morphological or molecular traits, or both [Kleinschmidt et al., 1986; Lavergne et al., 1996; Ozawa et al., 1997; Springer et al., 1997; de Jong, 1998; Shoshani and McKenna, 1998; Liu et al., 2001; Asher et al., 2003; Murata et al., 2003; Springer et al., 2004; Kemp, 2005; Kellogg et al., 2007; Ruiz-Herrera and Robinson, 2007; Gheerbrant, 2009; Nishihara et al., 2009; Cooper et al., 2014]. Hyraxes appear to share many physiological characteristics with extant Sirenia, including a low-quality food source and herbivorous lifestyle, poor thermoregulation, a relatively long gestation period and few offspring [Sale, 1970b; Olds and Shoshani, 1982; Rubsam et al., 1982]. The hyrax is also thought to share a sensory specialization otherwise present only in sirenians: a distribution of vibrissae (also known as tactile hairs or follicle-sinus complexes; FSCs) covering the entire body [Sale, 1970a]. These appear as single, long black hairs interspersed among the shorter pelage fur (fig. 1a). Whereas the hyrax has an ample distribution of pelage hair in addition to presumptive vibrissae, vibrissae represent the only hair type found in Sirenia [Reep et al., 2002], based on the criteria of the follicle and its affiliated dense innervation being surrounded by a blood sinus encased within a thick connective tissue capsule [Rice et al., 1986].

Mammalian vibrissae are generally restricted to specific regions of the body, most notably the face (e.g. the mystacial vibrissae of rodents). In contrast, the expanded vibrissal system in the hyrax would presumably greatly increase spatial resolution and allow navigation in the rocky crevices of the hyrax habitat where poor illumination might impair visual discrimination. Indeed, hyraxes often enter small caves by reversing into them and have poor visual acuity for near objects [Sale, 1970a]. The same author also noted that the hyrax’s presumptive vibrissae have a wide distribution with optimal positioning for displacement by external sources of the animal’s environment, including placement on the body regions of the anterior shoulder, carpal, back, flank and caudal regions [Sale, 1970a]. In dark, confined spaces, these hairs are stiffly erected, presumably to maximize contact with the surroundings [Sale, 1970a] and thus they appear to have a sensory function [Hvass, 1961]. Despite Sale’s account of the presence of presumptive vibrissae, no histological verification exists. Further accounts of hyraxes possessing presumptive vibrissae are provided by Sokolov and Sale [1981a, b; Sokolov, 1982] but also lack conclusive histological or immunohistochemical evidence classifying body hairs as vibrissae. Visual assessment confirms that single, long black hairs are interspersed among pelage fur in all four extant species of hyraxes.

Through systematic immunohistochemical analysis using anti-protein gene product 9.5 (PGP; a universal neuronal marker and cytoplasmic enzyme) as a standard in combination with antibodies against several other neural antigens, we were able to functionally characterize the innervation of hyrax vibrissal FSCs and to definitively determine for the first time that the long postfacial hairs on the hyrax body (fig. 1a) are in fact true vibrissae. Although the basic anatomical structure of FSCs remains relatively consistent across species, innervation patterns vary considerably under different evolutionary pressures and behavioral demands [Ebara et al., 2002]. Therefore, through the investigation of an evolutionary outlier, examination of the expanded vibrissal system in hyraxes also represents a critical contribution to the understanding of mammalian sensory systems overall.
Materials and Methods

Four adult hyraxes (P. capensis; 2 males and 2 females with weights ranging from 1.43 to 2.52 kg) were acquired from a licensed commercial supplier under IACUC protocol No. E252. The animals were fasted for 24 h prior to anesthesia. Anesthesia was induced briefly with 5% isoflurane in an induction chamber. The animal was then masked with 2% isoflurane and perfused through the left ventricle using phosphate-buffered heparinized saline (pH 7.0–7.4) at 37 °C followed by 4% phosphate-buffered paraformaldehyde. Hair follicle samples were acquired from 9 body regions (fig. 1b): mystacial, submental, shoulder and carpal; dorsal, lateral and ventral aspects of the mid-region, and both caudal and tarsal regions (nomenclature follows Sokolov and Kulikov [1987]). FSCs were sampled as previously described [Reep et al., 1998] using a No. 11 scalpel blade to extract a block of tissue (approx. 5 × 5 × 15 mm) surrounding the follicle of interest. Follicles were cut mediolongitudinally to facilitate fixation immediately following dissection and placed in 4% paraformaldehyde overnight. After 24 h of fixation the follicles were removed and placed in 0.1 M phosphate-buffered saline (PBS; pH 7.0–7.4) and 30% sucrose. Sections were cut using a cryostat. Sections for conventional epifluorescence evaluation were cut at 14 μm parallel to the long axis of the follicles or in cross-section (perpendicular to the long axis of the follicles), then directly thawed onto slides subbed with chrome-alum gelatin. They were allowed to air dry, then immunolabeled on the slides. Follicles for confocal analysis were cut at 80 μm and free-floating sections were immunolabeled before being mounted onto slides. After labeling, the slides were coverslipped using either 90% glycerin in PBS or Vectashield (Vector Laboratories).

The sections were processed for single and double immunolabeling with the following primary antibodies:

1. Anti-PGP (rabbit polyclonal, 1:800, catalog No. RA95101; UltraClone, Wellow, UK). The antigen was human PGP 9.5 protein purified from pathogen-free human brain. The antibody shows one band at 26–28 kDa on Western blot (tested in rabbits [Wilkinson et al., 1989]). Anti-PGP has been found to be a universal neuronal marker in other species, including rats [Fundin et al., 1997a; Rice et al., 1997], raccoons [Rice and Rasmusson, 2000], monkeys [Pare et al., 2001, 2002], naked mole-rats [Park et al., 2003], humans [Albrecht et al., 2006] and Florida manatees [Sarko et al., 2007]. Controls for the specificity of PGP labeling were provided in these species by the use of preadsorption controls and the demonstration that labeling was limited to neuronal innervation and glia. Staining in hyrax follicle sections produced a pattern of immunoreactivity that was identical to descriptions in the previous studies of the other species listed above.

2. Anti-neurofilament 200-kDa subunit (NF200; rabbit polyclonal, 1:800, catalog No. AB1982, lot No. 24080051; Chemicon International Inc., Temecula, Calif., USA). The antigen was a highly purified bovine neurofilament polypeptide. The antibody labels phosphorylated and nonphosphorylated NF200 and shows a band at 200 kDa and bands around 170–180 kDa on Western blot (tested in rabbits; manufacturer’s technical information). Immunolabeling for this protein has been localized to myelinated fibers, including Aβ and Aδ fibers, and Merkel endings in a variety of species, including rats [Fundin et al., 1997a; Rice et al., 1997], raccoons [Rice and Rasmusson, 2000], monkeys [Pare et al., 2001, 2002], naked mole-rats [Park et al., 2003], humans [Albrecht et al., 2006] and manatees [Sarko et al., 2007]. Con-
3. Anti-calcitonin gene-related peptide (CGRP; guinea pig polyclonal, 1:400, catalog No. T-5027, lot No. 061121; Peninsula Laboratories Inc., San Carlos, Calif., USA). The antigen is human α-CGRP with the following sequence: H-Ala-Cys-Asp-Thr-Ala-Thr-Cys-Val-Thr-His-Arg-Leu-Ala-Gly-Gly-Leu-Leu-Ser-Gly-Gly-Val-Lys-Asn-Asn-Phe-Val-Pro-Thr-Asn-Val-Gly-Ser-Lys-Ala-Phe-NH2. The antibody has 100% reactivity with human and rat α-CGRP, human CGRP (8–37), chicken CGRP and human β-CGRP. It has 0.04% cross reactivity with human amylin and 0.0% cross reactivity with rat amylin and human and salmon calcitonin (ELISA; manufacturer’s technical information). Although Western blot information was not available, this protein has been localized to Merkel cells, C-fiber innervation and vascular innervation in rats [Rosenfeld et al., 1983; Fundin et al., 1997a; Rice et al., 1997], and staining specificity has also been previously characterized in raccoons [Rice and Rasmusson, 2000], monkeys [Pare et al., 2001, 2002], naked mole-rats [Park et al., 2003], humans [Albrecht et al., 2006] and manatees [Sarko et al., 2007]. Controls for the specificity of CGRP labeling were provided in these species by the use of preadsorption controls and the demonstration that labeling was characteristic of Merkel cells, C-fiber innervation and vascular innervation. Staining in hyrax follicle sections produced a pattern of immunoreactivity that was identical to descriptions in the previous studies of the other species listed above.

4. Anti-S-100 (rabbit polyclonal, used neat, catalog No. 8200-0184, lot No. A2255; Biogenesis Inc., Brentwood, N.H., USA). The antigen was purified bovine S-100 protein. Although Western blot information was not available, this protein has been localized to brain glial cells and ependymal cells, in addition to Schwann cells of the peripheral nervous system, and the antibody has been found to have no cross-reactivity in rats (manufacturer’s technical information) [Moore, 1965, 1982; Stefansson et al., 1982]. Controls for the specificity of S-100 labeling have been provided in other species, including rats [Fundin et al., 1997a; Rice et al., 1997] and monkeys [Pare et al., 2001, 2002] by the use of preadsorption controls and the demonstration that labeling was limited to glia. Staining in hyrax follicle sections produced a pattern of immunoreactivity that was identical to descriptions in the previous studies of the other species listed above.

5. Anti-BNaC1α (rabbit polyclonal, 1:500; a gift from Dr. Jaime García-Anoveros). The antigen was N-terminal peptide MDLKESEPSEGLQPSSC (corresponding to residues 1–16 of mouse, rat and human BNaC1α [García-Anoveros et al., 2001]) and shows a band at 58.5 kDa on Western blot in rats and mice [García-Anoveros et al., 2001]. This protein is associated with low-threshold mechanoreceptors [García-Anoveros et al., 2001]. Controls for the specificity of BNaC1α labeling were provided in these species by the use of preadsorption controls and the demonstration that labeling was limited to low-threshold mechanoreceptors. Staining in hyrax follicle sections produced a pattern of immunoreactivity that appeared identical to descriptions in the previous studies of the other species listed above.
(For legend see next page.)
that emanate from FSCs as shown schematically in figure 2 and immunohistochemically in figure 3. The FSCs each consisted of a follicle core, affiliated dense innervation, and a surrounding blood sinus enclosed within a thick connective tissue capsule [Rice et al., 1986] along with a prominent ringwulst. Although the gross morphology of mystacial FSCs exhibited pronounced elongation compared to nonmystacial FSCs, similar innervation patterns were present in the FSCs across all body regions (fig. 2, 3). The FSCs from all body regions were innervated by two deep vibrissal nerves (DVNs) that penetrated the thick FSC capsule bilaterally at the cavernous sinus (CS) level and supplied sensory endings primarily to the ring sinus level. Several superficial vibrissal nerves supplied innervation primarily to the rete ridge collar (RRC) as well as the outer and inner conical body (ICB) levels of each FSC in addition to innervating adjacent skin and guard hairs. As observed in manatees, no papillary muscle slings – which are present in most other species examined to date – were observed in association with any of the hyrax FSCs, including those on the mystacial pad.

The dermis surrounding the capsule of each hyrax FSC contained numerous blood vessels that were densely innervated by a variety of thin-caliber fibers that either labeled with anti-NPY (and therefore constituted presumptive sympathetic innervation) or that expressed CGRP immunoreactivity (fig. 2, 4a, b). The CGRP-positive vascular innervation included both NF200-negative and NF200-positive thin-caliber fibers that were presumptive C and Aδ fibers, respectively, and were presumably sensory. Peptidergic (CGRP-positive, NF200-negative) and nonpeptidergic (CGRP-negative and NF200-negative) C-fiber innervation was also associated with each FSC at the RRC level.

**Innervation to the Mouth of the FSCs and Surrounding Epidermis**

The hyrax skin and RRC (the invagination of the skin toward the FSC) exhibited moderate innervation penetrating the epidermis, including thin-caliber fibers, most of which were NF200-negative and some of which were either CGRP-positive or CGRP-negative (fig. 4c–e). The remaining fibers labeled for both anti-PGP and anti-NF200. It has been shown in other species that NF200-negative fibers are characterized as thin-caliber, MBP-negative fibers, indicating that they are C fibers. In contrast, NF200-positive fibers can be thin or thick-caliber and consistently colabel for anti-MBP, indicating that they are Aδ or Aβ fibers, respectively [Fundin et al., 1997a; Pare et al., 2001; Rice and Rasmusson, 2000; Albrecht et

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**Fig. 2.** Schematic illustration of representative mystacial and postfacial hyrax FSCs characterizing the innervation types and sensory nerve endings observed. Labels for the mystacial FSC also apply to the postfacial FSC. Each is characterized by a dense connective tissue capsule, dense innervation (particularly MECs), a circumferential ring sinus, a prominent ringwulst and a dense mesenchymal sheath. The vibrissa of the mystacial FSC (but not the postfacial FSC) exhibited a ridged appearance at the level of the lower ring sinus continuing through the majority of the CS. The relative scale of each FSC is approximately accurate, but innervation is disproportionately scaled to optimize visualization. Scale bar = 1 mm. BM = Basement membrane, IRS = inner root sheath, MB = mesenchymal bulge, MS = mesenchymal sheath, ORS = outer root sheath, RS = ring sinus, RW = ringwulst, SG = sebaceous gland, SVN = superficial vibrissal nerve, VEN = venous supply.
al., 2006]. Thus, the majority of the innervation to the rete ridge of the FSCs included presumptive free nerve endings (FNEs) associated with C fibers, in addition to a lesser degree of Aδ fiber innervation (fig. 5a). No Pacinian corpuscles or Meissner corpuscles were observed. Numerous Merkel-ending complexes (MECs) were observed terminating at the RRC of hyrax FSCs and at the base of the adjacent epidermis in all areas (fig. 3a, d, 4c–e). These complexes consisted of Merkel endings, which colabeled with NF200 and PGP, and associated with Merkel cells,

Fig. 3. Longitudinal sections just off of the central axis of rock hyrax FSCs illustrating the overall structure, prominent ring sinus and ringwulst, and types of nerve endings present in facial and postfacial body regions. Mystacial (a), submental (b), lateral body (c) and ventral body region (d) FSCs exhibit the characteristics of true vibrissae. Arrowheads indicate mesenchymal bulges at the ring sinus level. The immunolabeling shown consisted of DAPI (blue, nuclear marker; 4',6-diamidino-2-phenylindole), NF200 (red) and PGP (green, universal neuronal marker). Scale bars = 300 μm (a–d). Cap = Capsule, Epi = epidermis, HP = hair papilla, MEs = Merkel endings, RS = ring sinus, RW = ringwulst, SG = sebaceous gland, TRB = trabeculae.

Color version available online
which colabeled with CGRP and PGP. An unusual feature of Merkel innervation in hyrax FSCs included semicircular dermal bulges laden with MECs at the RRC level (fig. 3a, d, 4c–e, 5a, b). These have not been seen in FSCs of any other species examined to date, but did resemble the Merkel innervated rete pegs in the glabrous skin of the raccoon [Rice and Rasmusson, 2000].

Progressing deep to the RRC, the connective tissue capsule of each FSC had a relatively narrow site of convergence (known as the outer conical body, or OCB) at the ‘neck’ of the FSC. Prominent circumferential sebaceous glands were evident at the OCB level (fig. 3a, d, 6a) and were particularly evident in mystacial, submental and carpal FSCs.

**Innervation to the ICB**

The OCB of the hyrax FSC enclosed a less compact region of connective tissue (the ICB; fig. 2–4). The ICB lev-
el of hyrax FSCs contained only a paucity of fine-caliber fibers that presumably terminated as FNEs (fig. 5b, 6b). The majority of these fine-caliber fibers labeled only with anti-PGP (presumptive sympathetic innervation) and a subset coexpressed CGRP or NF200 immunoreactivity (fig. 3b, 7a–c). No transverse lanceolate endings, such as those seen in rodent FSCs, were observed at the ICB level of hyrax FSCs across all the body regions sampled. There were also no trabeculae at the superficial end of the ring sinus, differing from the arrangement observed in manatees [Sarko et al., 2007].

**Innervation at the Ring Sinus Level**

Mystacial FSCs exhibited an unusual morphology with longitudinal ridges and grooves extending up from the CS to the lower ring sinus level. These were particularly evident in cross-sections through the FSC (fig. 6d, e). Not observed in any other species to date, these grooves were packed with axons ascending from the DVNs (fig. 6d, e).

Large-caliber Aβ fibers ascended from the DVN in each hyrax FSC to supply dense networks of MECs. MECs appeared to be uniformly distributed in the outer root sheath surrounding each follicle (fig. 3, 4c, 5e, 6c, 7e), la-

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**Fig. 5.** Confocal surface reconstructions illustrating the three-dimensional structure of representative hyrax FSC innervation and mechanoreceptors. 

- **a** Epidermal and RRC innervation, including small-caliber fibers and Merkel endings (submental FSC). 
- **b** Small-caliber innervation at the level of the ICB (mystacial FSC). 
- **c** Semicircular cusp of Merkel innervation at the level of the RRC (mystacial FSC). Arrowheads demarcate edges of the Merkel complex cusp. 
- **d** Extensive large-caliber innervation and Merkel endings at the level of the ring sinus (mystacial FSC). 
- **e** Large-caliber innervation and club endings at the level of the ring sinus (mystacial FSC). The immunolabeling shown consisted of PGP (universal neuronal marker).
beled positively for BNaC1α, and are known to be slowly adapting mechanoreceptors. Each Aβ fiber that terminated as Merkel endings branched extensively to form numerous endings that ramified over the circumference of the follicle (fig. 7b, c).

Each FSC had an unusually thick mesenchymal sheath (fig. 6b, c) coating the basement membrane of the follicle at the levels of the ring sinus and CS. A limited distribution of longitudinal lanceolate endings was observed at the upper ring sinus level (fig. 7d). In addition, 'tangle' endings, described previously only in Florida manatees [Sarko et al., 2007], were supplied by large-caliber DVN Aβ fibers and terminated within the mesenchymal sheath at the upper ring sinus/lower ICB level (fig. 7b, e). Though apparently smaller compared to those observed in manatees, each tangle ending had characteristic 'tangles' of NF200-positive fibers within a PGP-positive cytoplasmic envelope and was associated with terminal glia.

At the level of the ringwulst, mesenchymal bulges caused innervation from the DVN to curve around the bulges as it ascended superficially through the ring sinus level (fig. 3a, b, d, arrowheads, 7a–c, arrowheads). Club endings (unbranched terminations of Aβ fibers first described by Ebara et al. [2002]) were widely and uniformly spaced around the perimeter of the mesenchymal sheath at the level where the prominent, well-defined ringwulst was attached (fig. 3a, 5f, 7a, b). The mesenchymal sheath also contained a sparse distribution of thin-caliber fibers lacking morphologically distinct endings, presumably forming FNEs.

**Innervation at the CS Level and Hair Papilla**

No large caliber innervation was found within the trabeculated CS (fig. 3, 4c, 6d, e). Instead, innervation resided in closer proximity to the follicle along the mesenchymal sheath. As seen in other species, spiny endings were observed at the more superficial extent of the CS, near the ring sinus (fig. 3c, 8a), but were sparsely distributed as terminations of Aβ fibers. Reticular-like endings were also observed at this level, were associated with Aβ fibers, and appeared to project into pockets or papillae (fig. 3b, c, 8b). Finally, sparsely distributed spray endings of Aβ fibers were observed in the CS (fig. 8b). Spray endings, along with spiny and reticular endings, colabeled for NF200 and PGP. The hair papilla exhibited only sparse, fine-caliber innervation that was not superficially extensive (fig. 8c). This innervation included Aδ (NF200-positive/PGP-positive) fibers as well as C fibers (CGRP-positive/NF200-negative/PGP-positive) terminating as presumptive FNEs (fig. 8d, e).

**Guard Hairs**

Although not the focus of the present study (and therefore not characterized in detail), guard hairs proximal to vibrissae were observed (fig. 9). These hairs lacked the circumferential, blood-filled ring sinus and dense connective tissue capsule that characterize FSCs. Guard hairs were associated with prominent sebaceous glands and piloneural complexes that included circumferential longitudinal lanceolate endings which labeled for NF200, PGP and S-100 (fig. 9).

**Discussion**

**Innervation of FSCs**

The present report is the first to confirm that the expanded, body-wide distribution of elongated hairs, interspersed among pelage hair on the rock hyrax body, are in fact true vibrissae, exhibiting the definitive traits of a circumferential blood sinus, a variety of innervated mechanoreceptors and a dense connective tissue capsule [Rice et al., 1986]. MECs were the predominant form of the mechanoreceptor type found in hyrax FSCs. These were affiliated with large-caliber Aβ fibers ascending from the DVN to terminate predominantly in the outer root sheath at the ring sinus level. MECs exhibited two unusual features in rock hyraxes: (1) semicircular bulges laden with MECs that appeared to surround dermal ridges at the RRC level, and (2) mesenchymal bulges at the level of the ringwulst that were affiliated with MECs as innervation from the DVN ascended superficially through the ring sinus. Merkel endings are low-threshold, slowly adapting mechanoreceptors integral in detecting the compression as well as directionality of a stimulus during tactile discrimination [Iggo, 1963, 1966; Iggo and Muir, 1969; Munger et al., 1971; Gottschaldt and Vahe-Hinz, 1981; Johansson et al., 1982a, b; Johansson and Vallbo, 1983; Rice et al., 1986; Lichtenstein et al., 1990]. Merkel endings at the RRC are likely to detect large-angle hair deflections whereas endings at the ring sinus level presumably respond to more discrete perturbations, thus serving complementary roles based on where they are distributed within the FSC [Gottschaldt et al., 1973; Rice et al., 1986]. The dense distribution of MECs in FSCs across all body regions of the hyrax indicates a particular investment in directionality detection when a vibrissa is deflected [Burgess and Perl, 1973; Rice et al., 1986]. Similarly, MECs were the predominant type of mechanoreceptor present in manatee FSCs, although likely facilitating detection of underwa-
ter stimuli in the manatee’s case [Sarko et al., 2007] as opposed to detection of rocky crevice surroundings for the rock hyrax.

Club-like endings, present at the attachment site of the ringwulst, indicate sensitivity to mechanical perturbations at this location in hyrax FSCs. These mechanoreceptors were also present in manatees [Sarko et al., 2007] and likely respond to displacement of the ringwulst, situated within the blood-filled ring sinus, relative to the hair shaft of the follicle [Ebara et al., 2002]. In addition to club endings, a sparse distribution of longitudinal lanceolate endings was detected along the upper (more superficial) extent of the ring sinus. Lanceolate endings are low-threshold, rapidly adapting stretch receptors encoding aspects of vibrissal movement such as acceleration and deceleration of deflection [Burgess and Perl, 1973; Gottschaldt et al., 1973; Tuckett, 1978; Tuckett et al., 1978; Rice et al., 1986; Lichtenstein et al., 1990; Rice et al., 1997]. The low density of distribution for lanceolate endings and a high distribution of Merkel endings in both manatees [Sarko et al., 2007] and rock hyraxes emphasizes an investment in detecting the presence or absence of a stimulus – and the direction of origin of that stimulus – over dynamic stimulus properties such as speed of vibrissal deflection. However, dense palisades of longitudinal lanceolate endings were detected in pelage hair, indicating that these hairs are attuned to speed of displacement. Given these disparate mechanoreceptor distributions, vibrissae and pelage may serve complementary roles on the hyrax body. Presumably, initial detection of surrounding substrates (e.g. holes or rock crevices) or threats would first deflect elongated vibrissae that extend further from the body, signaling directionality of the contact. Subsequently, as the stimulus approaches more closely to the hyrax body, pelage hair deflection (in addition to large-angle deflection of vibrissae) might signal the speed of contact.

Fig. 6. Cross-sectional series from a hyrax mystacial FSCs, from superficial to deep levels. Prominent sebaceous glands are evident (a) and a dense connective tissue capsule surrounds the follicle and its affiliated dense innervation (b–f). Dense Merkel cell innervation can be seen at the ring sinus level surrounding the follicle circumferentially and terminating within the outer root sheath (c). The irregular morphology of the hair follicle becomes apparent at the lower ring sinus and CS levels where prominent longitudinal ridges appear (white arrowheads; d, e), and an absence of lateral innervation (within the trabeculae) at the CS level is evident (d–f). The immunolabelling shown consisted of PGP (universal neuronal marker). Scale bar = 300 μm (a–f). Cap = Capsule, GM = glassy membrane, HP = hair papilla, HS = hair shaft, MCs = Merkel cells, MS = mesenchymal sheath, ORS = outer root sheath, RS = ring sinus, RW = ringwulst, SG = sebaceous gland, TRB = trabeculae.

‘Tangle endings’, described previously only in Florida manatees [Sarko et al., 2007], were also present within rock hyrax FSCs. They terminated in similar locations (along the mesenchymal sheath of the upper ring sinus/ lower ICB level) and exhibited similar immunohistochemical characteristics as ‘tangles’ of NF200-positive fibers within a PGP-positive cytoplasmic envelope associated with glia. The rock hyrax tangle endings were notably smaller compared to those observed in the manatee, possibly due to relative scaling with body size or diminished function. In manatees, tangle endings were present in both facial and postfacial FSCs and were thought to enhance directionality detection [Sarko et al., 2007].

A second type of novel mechanoreceptor previously described only in manatee facial vibrissae [Sarko et al., 2007], the trabecular ending, was not detected in rock hyrax FSCs. These mechanoreceptors were thought to be an adaptation to detection of underwater stimuli, particularly in detecting tension along the trabeculae as the particularly rigid manatee facial vibrissae are deflected [Sarko et al., 2007]. In fact, innervation was absent in the rock hyrax FSC CS, and branches from the DVN terminated exclusively along the mesenchymal sheath. The absence of trabecular endings in rock hyraxes supports the hypothesis that these mechanoreceptors are an aquatic adaptation optimized to facilitate tactile exploration and object recognition. Instead, spiny and reticular endings – absent in manatees, but present in rock hyrax FSCs – may serve a related function. These endings have been noted in a wide range of species in the CS and are thought to detect stresses within the mesenchymal sheath parallel to the long axis of the follicle, in the case of spiny endings, or to detect tension perpendicular to the long axis of the follicle with directional sensitivity, in the case of reticular endings [Fundin et al., 1997b; Rice et al., 1997; Ebara et al., 2002].

Vibrissae: Comparative Distribution

In most terrestrial mammals vibrissae are found only on restricted regions of the body, and are particularly concentrated on the face [Pocock, 1914; Eisenberg, 1981]. Some rodents possess a few carpal, antebiachral or tarsal vibrissae [Fundin et al., 1995], and a few vibrissae are found on the trunk in two squirrel species [Hyvärinen et al., 1977; Sokolov and Kulikov, 1987]. Naked mole-rats exhibit an expanded system of body hairs that function as tactile hairs but are enlarged guard hairs lacking affiliated FSCs rather than true vibrissae [Crish et al., 2003]. In contrast, hyraxes exhibit an expanded, body-wide distribution of vibrissae interspersed among pelage fur.
Similar to terrestrial mammals, most marine mammals also exhibit a restricted distribution of vibrissae. Pinnipeds and cetaceans have vibrissae on the head only [Ling, 1977]. Pinnipeds exhibit morphological elaborations of the vibrissae that are associated with prey capture and benthic foraging [Marshall et al., 2006; Ginter et al., 2012]. Baleen whales have approximately 100 thin vibrissae on the upper and lower jaws, and some exhibit tubercles on the head that contain innervated hairs [Mercado, 2014]. In most odontocete cetaceans, hair is present only as prenatal vibrissae which atrophy, resulting in vibrissal crypts which are electroreceptive in some taxa [Ling, 1977; Czech-Damal et al., 2012, 2013]. An important exception is freshwater river dolphins, which have well-developed vibrissae along the upper and lower jaws that may be used in prey localization [Layne and Caldwell, 1964; Ling, 1977]. As first described by Dosch [1915] in dugongs and later verified in manatees, sirenians are unusual in having vibrissae distributed over the entire body, and this is the only type of hair they possess [Bryden et al., 1978; Kamiya and Yamasaki, 1981; Sokolov, 1986; Reep et al., 1998, 2001, 2002]. Marine mammals of the order Sirenia, among the hyrax’s closest extant relatives, are the only other species known to exhibit this elaborated pattern of true vibrissae distributed on the entire body [Reep et al., 2002; Sarko et al., 2011]. This elaboration of vibrissal distribution appears to have been adaptive enough to be conserved across the orders Sirenia and Hyracoidea.

**Vibrissae Evolution and Comparative Function**

Vibrissae facilitate a wide range of perceptual and behavioral functions, including spatial orientation and navigation in rodents [Sokolov and Kulikov, 1987; Brecht et al., 1997], object discrimination and prey detection in Australian water rats [Dehnhardt et al., 1999], social display in pinnipeds [Miller, 1975], tracking hydrodynamic trails in harbor seals [Dehnhardt et al., 2001] and oripulation in manatees [Marshall et al., 1998; Reep et al., 2001]. Hyraxes are the only terrestrial mammals with a body-wide distribution of vibrissae, although some rodents have several postfacial vibrissae associated with restricted body regions and likely used for spatial orientation (e.g. in arboreal environments). The principal utility of vibrissae in the rock hyrax may center around predator avoidance. As hyraxes are slow-moving and possess few defense mechanisms against predators (mainly leopards, snakes, caracals, wild dogs and eagles [Coe, 1962; Turner and Watson, 1965]), they appear to depend on rock crevices for safety, escape and survival [Fairall et al., 1983]. In addition, hyraxes will inhabit holes that they have dug for themselves in the ground [Shortridge, 1934] or that have been usurped from aardvarks or meerkats [Roberts, 1951]. These holes are often small – ‘no deeper than an adult hyrax in a crouching position’ – with entrances too small to permit entrance to the larger and more frequent predators [Sale, 1966]. Seasonal food availability – and a balance between foraging or grazing versus predation risk – may be an integral component as well. The low-quality and scarce food sources of the rock hyrax drive more frequent foraging at greater distances (>50 m) in the winter, increasing risk when the dense vegetative cover that is present in summer cannot be relied on in addition to close proximity to rock crevices [Brown and Downs, 2006]. Holes and crevices appear to offer not only protection, but also water retention, proximity to vegetation and climactic moderation that is somewhat sheltered from surrounding fluctuations [Turner and Watson, 1965]. Presumably, a distribution of vibrissae on the hyrax body would enhance detection of those protective boundaries within holes and crevices, guiding navigation and enhancing survival where visual cues are minimal. In Hebrew, rock hyraxes are known as ‘Shaphan’, translating as ‘the hidden one’ [Olds and Shoshani, 1982], and indeed hiding may be one of the rock hyrax’s greatest forms of defense, facilitated by tactile feedback through a large distribution of vibrissae.

**Fig. 7.** Characterization of hyrax FSC innervation at the ring sinus (RS) level (longitudinal planes of the section). a Characteristic innervation at the RS and CS levels of a representative FSC from the caudal body. Arrowhead denotes a mesenchymal bulge at the ring sinus level in proximity to the ringwulst (RW). Large-caliber fibers from the DVN can be seen ascending superficially through the CS, curving around the mesenchymal bulge to terminate as MECs. b At the level of the ring sinus, club endings and MEC networks can be observed. A mesenchymal bulge at the ring sinus level in proximity to the ringwulst is visible (arrowheads), and presumptive ‘tangle’ endings are apparent at the upper RS/lower ICB level as terminations of large-caliber fibers. c Fine-caliber innervation is observed at the outer and ICB levels, whereas the ring sinus is characterized by dense, circumferential networks of MECs. The mesenchymal bulge proximal to the ringwulst is denoted by the arrowhead. d Longitudinal lanceolate endings were present along the mesenchymal sheath at the upper extent of the ring sinus, but were relatively sparsely distributed. e MECs terminate in the outer root sheath of the follicle at the ring sinus level, and large tangle endings were observed. Scale bars = 300 μm (a-d), 75 μm (e). Cap = Capsule, HP = hair papilla, LLEs = longitudinal lanceolate endings, RS = ring sinus, TRB = trabeculae.
Evolutionary Considerations of an Expanded Vibrissal System

Any theory concerning hyrax vibrissae must take into account that all four extant species appear to exhibit vibrissae (based on visual assessment, although no anatomical or immunohistochemical confirmation has been made in any hyrax species prior to the present study). Tree hyraxes (Dendrohyrax) are largely nocturnal and – as their name suggests – predominantly live in tree habitats, commonly nesting in the hollows of Hagenia trees.
[Milner and Harris, 1999]. Other terrestrial mammals with nonfacial vibrissae are also arboreal, including squirrels [Hyvärinen et al., 1977], with vibrissae presumably providing feedback regarding body position relative to the substrate (tree branches or trunks) [Sokolov and Kulikov, 1987]. All hyrax species are known to be skilled at climbing and jumping [Rubsamen et al., 1982], presumably involving proprioceptive feedback facilitated by the vibrissae position on the postfacial body. The yellow-spotted rock hyrax (H. brucei) habitat consists of boulders, cliffs and small hills, with this species often occupying the same rock crevices as P. capensis [Hoeck, 1989; Barry and Shoshani, 2000] (although minimal overlap in their diets minimizes competition for food resources [Kingdon, 1971]). Presumably postfacial vibrissae would confer similar advantages (as described above) navigating these overlapping habitats across rock hyrax species. Beyond feedback related to immediate surroundings (tree hollows, holes in the ground or rocky crevices), vibrissae also likely provide a sensitive warning system to threats. Since vibrissae extend to a greater length from the body, immediate detection of contact with an object, predator or conspecific (a high level of intraspecific aggression is present in hyraxes) would allow the hyrax to more quickly flee danger or orient toward it and attack.

Given that hyraxes possess vibrissae in addition to pelage hair on the entire body, that sirenians (manatees and dugongs) exhibit vibrissae on the entire body as the only hair type present [Reep et al., 2002], and that elephant vibrissae have been discovered on the tip of the trunk [Rasmussen and Munger, 1996] but not on the body [Reep, unpubl. assessment of histological sections], we can hypothesize that a distributed system of vibrissae on the entire postfacial body, in addition to the face, constitutes the ancestral condition for the supraorder Paenungulata. Across mammalian phylogeny, evidence for the evolution of pelage hair and vibrissae is difficult to trace due to the absence of soft tissue in the fossil record, but evidence of hairs can be traced back to multituberculates in the Late Paleocene, and hairs are thought to have been present in the most recent common ancestor of multituberculates, monotremes and therians approximately 210 million years ago [Meng and Wyss, 1997]. Vibrissae are thought to be phylogenetically older than pelage hair [Watson, 1931; Brink, 1956; Estes, 1961; Tatarinov, 1967; Findlay, 1968, 1970] and generally develop first during

Fig. 9. Hyrax guard hair innervation. In contrast to FSCs, guard hairs lacked a circumferential ring sinus and exhibited only limited density and types of innervation. This innervation was characterized by piloneural complexes that included circumferential longitudinal lanceolate endings: longitudinal plane of the section (a), oblique plane of the section (b). The immunolabeling shown consisted of NF200 (red) paired with PGP (green, universal neuronal marker) or S-100 (green, glial and ependymal marker). Scale bars = 150 μm. Epi = Epidermis, HS = hair shaft, LLEs = longitudinal lanceolate endings.
ontogeny [Grüneberg, 1943; Davidson and Hardy, 1952; Dun, 1959; Kollar, 1970; Ling, 1977; Ahl, 1986; Klauer et al., 2001]. Maderson [2003] proposed that mutations in patterning genes led to multiplication of protovibrissae and protopelage, conferring the selective advantages of protecting the skin from abrasion and enhancing thermoregulation (minimizing cutaneous water loss and providing an insulator barrier). A mutation leading to up-regulation of Wnt/β-catenin is a likely candidate [Maderson, 2003; Dhouverlly, 2009], given that β-catenin causes follicle morphogenesis [Gat et al., 1998; Moore and Lemischka, 2006] and Wnt initiates hair follicle development, also helping to determine spacing between hair follicles [Gat et al., 1998; Andl et al., 2002; Chang et al., 2004; Sick et al., 2006]. In addition, Blimp 1 – a zinc-finger transcriptional repressor – specifically induces vibrissae [Robertson et al., 2007]. Such cell signaling mechanisms point to substrates that were likely to have been modified in Hyracoidea and Sirenia lineages to produce an expanded system of vibrissae, ultimately facilitating navigation of the environment and enhancing survival.

Peripheral specializations that involve dense innervation, such as vibrissae, are reflected by enlarged represen-

tations within the central nervous system. Microelectrode mapping of the rock hyrax primary somatosensory cortex mirrors physiological specializations in the periphery by demonstrating enlarged perioral and intraoral represen-
tations [Welker and Carlson, 1976]. In fact, 67% of the primary somatosensory cortex was taken up by the head representation, an expansion thought to facilitate tactile feedback during grazing on vegetation [Welker and Carlson, 1976] similar to sheep [Johnson et al., 1974]. In closely related taxa such as manatees, the perioral region is also believed to be represented by large cortical regions [Reep et al., 1989; Marshall and Reep, 1995; Sarko and Reep, 2007] further indicating conserved anatomical and physiological traits for Hyracoidea and Sirenia.

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