A new type of somatosensory organ in the nasolabial skin of the dog

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Abstract
A new morphological type of somatosensory organ is described. It is found in the glabrous skin of the dog nose (rhinarium or planum nasale) and situated in dermis papillae. The otherwise thick epidermis forms a thin window above the organ. There are only a few layers of keratinocytes in the window and the corneocytes are much thinner than elsewhere. The organ consists of highly branching cells that wrap naked nerve endings emanating from myelinated nerve fibers originating in the outer dermal nerve plexus. The structure entirely fills the top of the dermal papilla. The intercellular spaces of the organ and its surroundings are occupied by an extended areolar basal lamina.

KEYWORDS
dogs, electron microscopy, glabrous skin, somatosensory organ

1 INTRODUCTION
Mammalian skin has time-honored sensory organs: Vater-Pacini corpuscles (Pacini, 1835; Vater, 1741), Meissner corpuscles (Wagner & Meissner, 1852), Krause endings (Krause, 1860), Merkel cell-neurite complex (Merkel, 1875; Ramirez et al., 2015), Ruffini corpuscles (Ruffini, 1893), and Golgi-Mazzoni corpuscles (Ruffini, 1893). All gathered under the term sensory nerve formations (Malinovsky, 1996). A multitude of investigations has studied their structures and functions. Furthermore, they have been the subject of several reviews (e.g., Birder & Perl, 1994; Chouchkov, 1978; Iggo & Andres, 1982; Martin-Alguacil, de Gaspar, Schober, & Pfaff, 2016; McGlone & Reilly, 2010; Munger & Ide, 1988). Sensory nerve formations are characterized with respect to the position in the skin and are anatomically distinct end organs. The Merkel cell-neurite complexes are the only one situated in the epidermis. The remaining structures are confined to the dermis and they all have a capsule of non-nervous origin. The corpuscles and Merkel cell-neurite complexes react to tactile stimuli.

Besides the defined organs, unmyelinated nerve endings penetrate the epidermis and end, usually with a terminal knob, below the stratum corneum (Alvarez et al., 1988; Elofsson, Tuminate, & Kröger, 2015; Malkock et al., 2012; Montagna, Roman, & Macpherson, 1975). Unmyelinated free nerve fibers are also found in the dermis (Cauna, 1973, 1980; Silverman, Munger, & Halata, 1986). Due to lack of additional morphological features, free nerve endings are not separated into categories, even though they may have separate functions. One exception is the unmyelinated, epidermal nerve endings of the Eimer’s organ (Eimer, 1871) in lemurs, which have spines penetrating keratinocytes. (Elofsson et al., 2015)

The function of the specialized skin on the rhinarium or planum nasale is known only in a few cases (e.g., Catania, 2000) and seems to vary between species (Barker & Welker, 1969). Earlier morphological studies on the skin of the rhinarium in a variety of species have revealed new and surprising information (Elofsson et al., 2015; Elofsson & Kröger, 2017; Elofsson & Kröger, 2018; Elofsson, Tuminaite, & Kröger, 2016), as has a study on the temperature dynamics of the dog nose (Kröger & Goiricelaya, 2017). Structural and functional studies must complement each other for an understanding of an organ and therefore we launched the current study when we had the opportunity to study rhinurium skin material from domestic dogs and dingoes,
which are descendants of domestic dogs brought to Australia a few 1000 years ago (Oskarsson et al., 2011).

The thick epidermis of the skin on the dog rhinarium has an undulating border towards the dermis. Ridges and cones of the epidermis extend into the dermis. They are separated by papillae of dermis approaching the skin surface. A specific feature of the corneal layer of the nasolabial skin is the thickness of the corneocytes. They can be up to 5 μm thick and are spindle-shaped in cross section (fig. 5c in Elofsson et al., 2016).

Merkel cell-neurite complexes, Vater-Pacini corpuscles, and unmyelinated nerve endings in the epidermis are the “classical” sense organs present in the dog rhinarium skin. A new type of sensory receptor was found in the dog rhinarium and is described below.

2 | MATERIAL AND METHODS

The investigated dogs belong to the subspecies Canis lupus dingo Meyer, 1793 and Canis lupus familiaris Linnaeus, 1758. One female (11 years) and one male (8 years) dingo, sacrificed for illness and inbreeding, were provided by Öland Zoo (Ölands djurpark) in Sweden (department protocol BI 2017, CLD M, CLD F). The dogs include specimens of golden retriever (male 11 years), and mixed breed (two males, 7 and 9 years) removed for illness and age (department protocol BI 2016, GR M, MB M 1,2). The dog specimens were supplied by the University of Veterinary Medicine, Vienna, Austria.

Punch biopsies, 3 or 4 mm in diameter, were secured from the frontal part of the rhinarium three on both sides of the midline. The biopsies were divided and immersed in 2.5% or 3% glutaraldehyde (Agar Scientific, Stansted, UK) in sodium cacodylate buffer 0.15 mol L$^{-1}$ (pH 7.2–7.3) (Agar Scientific) for 3 hr. After fixation, the preparations were rinsed repeatedly in buffer before further processing in 1% Os O$_4$ (Agar Scientific) in 0.15 mol L$^{-1}$ sodium cacodylate buffer for 1 hour at room temperature (about 20°C). After rinsing in the same buffer, the preparations were dehydrated in a graded ethanol series, transferred to acetone and then Epon (Agar Scientific). The Epon was polymerized at 60°C for 48 hr.

Thin sections of specimens (2 μm) for light microscopy, previously prepared for electron microscopy and treated as above, were stained in a mixture of methylene and Azur II blue. Ultrathin continuous sections were cut with a Leica Ultracut UCT microtome (Leica Microsystems GmbH, Wetzlar, Germany). Staining on copper grids was performed with 2% uranyl acetate (Ted Pella, Redding, CA) and 1% lead citrate (Merck KGaA, Darmstadt, Germany) for 30 min. Sections were studied in a JEOL 1240 Plus (Jeol, Tokyo, Japan).

3 | RESULTS

The new organ is found distally in the longest dermal papillae, one or a few in each of the cushions constituting the rhinoglyphic pattern (Figure 1). A rhinoglyphic pattern of epidermal domes is present in several mammalian orders (Hill, 1948). The sizes of these cushions or domes vary between dog breeds and sizes (own observations). In the dogs we studied, each dome was approximately 1 mm in diameter. The epidermis above the organ is thin and forms a little window with a width of about 50 μm in the otherwise thick epidermis. The corneal cells change shape and become thin (between 0.5 and 1 μm; Figure 2a). About 10 corneal cells are layered upon each other. There is one single granular layer and only one to three spinous cell layers. In consequence, the organ is situated only approximately 25 μm from the skin surface. These small windows can be observed in the light microscope (Figure 2a, inset).

The organ consists of naked nerve endings wrapped in cells, here called supporting cells. The supporting cells have a heavily folded surface forming thin membranes that wrap around and enclose the nerve terminals (Figure 2b). The entire naked portion of the nerve terminals is in contact with the supporting cells. The supporting cell cytoplasm is compact with many free ribosomes, sites with rough endoplasmic reticulum, centriole, mitochondria, microtubules, and irregular dark bodies. The surface of the supporting cells displays an ongoing endocytotic process with invaginating pinocytotic vesicles. The enclosed vesicles are frequent in the periphery of the cell but also present inside the cell (Figure 2c,d). The cell bodies with nuclei of the supporting cells are situated mostly in the periphery of the structure formed by the organ (Figure 3a). The supporting cells bodies are more numerous distally in the organ. Further proximal, in the organ only the branches of the supporting cells wrap the nerve terminals (Figure 3b).

The nerve fibers of the organ originate in the outer dermal nerve plexus. Basally in the dermal papillae, the fibers are combined in a small bundle surrounded by fibrocytes, forming a tube (Figure 3c). The myelin sheaths of the nerve fibers in the tube contains between 15 and 20 layers. The fiber diameter varies between 1 and 2 μm. The tube ends distally in the papilla and the nerve fibers fan out towards the epidermis. The nerve fibers lose their thick myelin sheaths outside...
the tube and only one Schwann cell covers the nerve fiber for a short distance. The axons divide repeatedly and lose the Schwann cells, which are replaced by supporting cells enveloping the terminal axon branches (Figure 4a).

The axons branches and the supporting cells form an intricate cylindrical structure of strings, approximately 100 μm in length, which floats free in the tip of the dermal papilla and with the organ tip close to the epidermal window (Figure 4b). Distally, the papilla narrows around the organ and the tip of the organ bends 90° and runs parallel to the skin surface for a short distance (Figures 1 and 2a). The interior of the string cylinder and the space surrounding it consists of a thick basal lamina, formed by the supporting cells that merge with that of the epidermis. The basal lamina has darker and lighter portions which sometimes form patterns. The interior and surroundings of the organ lack fibrocytes, connective fiber bundles, and capillaries.

4 | DISCUSSION

The lack of a capsule distinguishes the new dermal sensory organ from other dermal receptors. All previously investigated dermal sensory organs are surrounded by a capsule, which is important for their mechanoreceptive function. The capsule is composed of three structures: an outer and inner subcapsule separated by an intermediate layer.

The outer subcapsule is cellular and the cells are arranged in circular lamellae varying from a few to many. The cellular portion of the
capsules consists of fibrocytes and flattened Schwann cell lamellae. The Meissner corpuscles deviate by having an incomplete outer capsule consisting mostly of collagen fibers which also replace the space. The intermediate layer between the subcapsules contains mainly collagen fibers and fibrocytes. The space is small or indistinct in Krause (Cavalotti, Malinovsky, & D’Andrea, 1995; Chouchkov, 1973; Spassova, 1974), Vater-Pacini (Pease & Quilliam, 1957), and Golgi-Mazzoni (Chouchkov, 1973) corpuscles, large in Ruffini (Halata & Munger, 1981), corpuscles.

The inner subcapsule is also cellular and appears as a few or several layers. In the Ruffini corpuscles, the collagen fibers in the inner capsule fuse with those outside (Chouchkov, 1978; Iggo & Andres, 1982). In the Meissner corpuscles, the flattened cells of the inner capsule are arranged in horizontal stacks and the axons terminals weaves back and forth between the lamellae (Cauna & Ross, 1960; Munger & Ide, 1988).

The mechanoreceptive function of the cutaneous receptors has been established in a number of investigations (Biemesderfer, Munger, Binck, & Dubner, 1978; Chambers, Andres, Von Düring, & Iggo, 1972; Iggo & Andres, 1982; Iggo & Muir, 1969; Iggo & Ogawa, 1977; Quindien, Lai, & Barocas, 2015). For this function, it is important that the connective tissue and collagen fibers are part of the capsule and linked to the surrounding connective tissue. (Pawson et al., 2009). The function as mechanoreceptors may be complemented by additional capacities reported in more recent investigations (Owens & Lumpkin, 2014; Paré, Mazurkiewicz, Smith, & Rice, 2001).

**FIGURE 3** (a) Dingo. Cross section distally through a dermal papilla. The dog organ (star) surrounded by nuclei of the supporting cells (arrows). Scale bar 10 μm. (b) Dingo. Cross-section of a dermal papilla further proximally (compared to (a)) with the dog organ (star). Note the fewer nuclei of the supporting cells. Scale bar 10 μm. (c) Dog, golden retriever. A bundle of nerve axons (arrows) running inside a dermal papilla to the dog organ (not shown). Epidermal surface to the top. Scale bar 10 μm
In strong contrast to the mechanoreceptors, the dog organ lacks a capsule and collagen fibers inside or in the immediate neighborhood. Instead, there are supporting cells that form an inseparable unit with the nerve terminals. The nature of the cells termed supporting cells here is uncertain. These centriole-containing cells have no immediate similarity with Schwann cells and could be either transformed such cells or have another origin. They invest the axon branches closely and a part of their function could be a scaffold for the many axon branches. The cells probably have a low synthetic production, but high endocytotic activity, forming a multitude of pinocytotic vesicles. They may have a function in transducing stimuli of some kind. The organ is similar in the dingoes and different dog breeds used in this investigation. Dingoes and domestic dogs are so closely related (e.g., Oskarsson et al., 2011) that differences were not to be expected.

The new dermal sensory organ shares the same type of innervation with the mechanoreceptive organs. Nerve fibers from the dermal nerve plexus lose their Schwann cell clothing before the entrance into the organs. The nerve terminals of the mechanoreceptive organs may have one or more branches (Paré et al., 2001), whereas the new dermal sensory organ of dogs has widely branching terminals.

The axons from the outer dermal nerve plexus reaching to the dog organ fit with regard to axon diameter and myelination into the category of A δ (Martin-Alguacil et al., 2016). These fibers together with C-fibers, distributed on the skin surface, are found in receptors that relay painful, cold and thermal information (Alamri, Wood, Ivanusic, & Brock, 2018; McGlone & Reilly, 2010; Schepers & Ringkamp, 2010; Vriens, Nilius, & Voets, 2014). The myelinated axons of the dog organ secure a relatively fast conductance.

The organ is situated close to the skin surface in a dermal papilla and has a specific thin area of epidermis above it. The thick corneocytes was shown to be a specific feature of the epidermis of rhinaria (Elofsson et al., 2016). The thin corneocytes above the new dermal sensory organ are thus an adaptation to the presence of the organ. The position and thin barrier make the organ a candidate for a sensory organ. Since its morphology is distinctly different from known mammalian mechanosensory skin organs, a role as a receptor for tactile stimuli is less probable. The organ is absent in other carefully studied rhinaria, such as in moles and their relatives (Catania, 2000) and in lemurs (Elofsson et al., 2015). It is interesting in this context that carnivorans have lower rhinarius temperatures than other mammalian groups (Gläser & Kröger, 2017). Dogs in particular have intricate rhinarius temperature dynamics (Kröger & Goiricelaya, 2017). A temperature receptor could thus be an option for the function of the organ described here.

ACKNOWLEDGMENTS
We are grateful to Karl-Johan Nordfeldt and Öland zoo for supplying the dingoes and to Michael Leschnik at the University of Veterinary Medicine in Vienna for the opportunity to get the biopsies from dogs. Inga Tuminaite secured the preparations from Vienna, Rita Wallén and Ola Gustafsson were of invaluable technical support. Morgan Luce checked the language. The Royal Physiographic Society in Lund financially contributed to the project.

CONFLICT OF INTEREST
No conflict of interest to declare.

DATA AVAILABILITY STATEMENT
Data sharing is not applicable to this article as no new data were created or analyzed in this study.

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