Morphological and histological features of the vomeronasal organ in the brown bear

Short running page heading: Vomeronasal organ in bear

Jumpei Tomiyasu[1, 2], Daisuke Kondoh[3]*, Hideyuki Sakamoto[4], Naoya Matsumoto[4], Sasaki Motoki[2, 3], Kitamura Nobuo[2, 3], Shingo Haneda[1] and Motozumi Matsui[1, 2]

[1]Laboratory of Theriogenology, Department of Applied Veterinary Medicine, Obihiro University of Agriculture and Veterinary Medicine, Obihiro, Hokkaido 080-8555, Japan

[2]The united Graduate School of Veterinary Sciences, Gifu University, Gifu, Gifu 501-1193, Japan

[3]Laboratory of Veterinary Anatomy, Department of Basic Veterinary Medicine, Obihiro University of Agriculture and Veterinary Medicine, Obihiro, Hokkaido 080-8555, Japan

[4]Noboribetsu Bear Park, Noboribetsu, Hokkaido 059-0551, Japan

*Corresponding author

Author: Daisuke Kondoh

Tel: +81-155-49-5369

Email: kondoh-d@obihiro.ac.jp

Address: Nishi 2-11, Inada-cho, Obihiro, Hokkaido 080-8555, Japan

Abstract

The vomeronasal organ (VNO) is a peripheral receptor structure that is involved in reproductive behavior and is part of the vomeronasal system. Male bears exhibit flehmen behavior that is regarded as the uptake of pheromones into the VNO to detect estrus in females. However, the morphological and histological features of the VNO in bears have not been comprehensively studied. The present study investigated the properties and degree of development of the VNO of the brown bear by histological, histochemical and ultrastructural methods. The VNO of bears is located at the same position as that of many other mammals, and it opens to the mouth like the VNO of most carnivores. The shape of the vomeronasal cartilages and the histological features of the sensory epithelium in the bear VNO are essentially similar to those of dogs. Receptor cells in the VNO of the bear possess both cilia and microvilli like those of dogs. The dendritic knobs of receptor cells were positive for anti-G protein alpha-i2 subunit ($G_{\alpha i2}$) but negative for anti-G protein alpha-o subunit, indicating preferential use of the V1R-$G_{\alpha i2}$ pathway in the vomeronasal system of bears, as in other carnivores. The VNO of the bear possessed three types of secretory cells (secretory cells of the vomeronasal gland, multicellular intraepithelial gland cells and goblet cells), and the present findings showed that the properties of secretory granules in these cells were also various. The vomeronasal lumen at the middle region of the VNO invaginated toward the ventral region, and this invagination contained tightly-packed multicellular intraepithelial gland cells. To our knowledge, this invagination and intraepithelial gland masses in the VNO are unique features of brown bears. The VNO in the brown bear, especially the secretory system, is morphologically well-developed, suggesting that this organ is significant for information transmission in this species.

45 Keywords: vomeronasal glands, pheromones, reproductive behavior, olfactory
46 communication

Introduction

The primary form of communication in many mammalian species is olfactory (Müller-Schwarze, 2006). Olfaction is mediated by the main olfactory system, and by the vomeronasal system that mainly receives pheromones and is associated with changes in reproductive behavior (Wysocki, 1979). The vomeronasal organ (VNO) is the peripheral receptor organ of the vomeronasal system and it projects into the accessory olfactory bulb (McCotter, 1912). The VNO of most mammals comprises cartilage and soft tissue that contains a lumen, veins, arteries, glands and nerve bundles, and the vomeronasal lumen is medially and laterally covered by vomeronasal sensory (VNE) and non-sensory (NSE) epithelia, respectively (Halpern, 1987). Mucosal fluid secreted by the vomeronasal galnds on luminal surface of the VNO is associated with the detection of odorants by receptor cells (Khew-Goodall et al., 1991).

The vomeronasal receptors comprise the type 1 family (V1Rs) coupled with G protein α-i2 subunit ($G_{\alpha i2}$) and type 2 family (V2Rs) coupled with G protein α-o subunit ($G_{\alpha o}$) (Dulac & Axel, 1995; Herrada & Dulac, 1997; Matsunami & Buck, 1997; Ryba & Tirindelli, 1997), and the expression of these receptor types in the VNO varies among animal species. It is considered that results of immunohistochemistry against anti-G protein alpha subunits reflect the receptor families expressed in the vomeronasal system.

The family Ursidae includes polar, American black, Asiatic black, brown, spectacled, sun and sloth bears, and the giant panda. They are generally solitary, and some of them have a wide home range (Polar bear: Ferguson et al., 1999; Brown bear: Dahle & Swenson, 2003; American black bear: Koehler & Pierce, 2003; Asiatic black bear; Hwang et al., 2010, Spectacled bear: Castellanos, 2011). As odorants persists for long periods even in the absence of the producer, the vomeronasal system may be a suitable mechanism for

transmission of information in bears. In fact, male polar bears seem to follow single sets of tracks with flehmen behaviors (curling their upper lips and exposing the front gums) to mate with females (Stirling et al., 2016), and they distinguish sex according to their pedal scents (Owen et al., 2015). In addition, male American black bears also show flehmen behaviors during anogenital and excremental investigations (Gonzales et al., 2013). Therefore, chemosensory communication mediated by the VNO apparently functions, in part to determine the status of estrus in female bears.

The VNO has been topographically determined in Asiatic black and American black bears among the Ursidae (Befu, 2009; Kilham, 2014). The VNO of the Asiatic black bear seems to possess the same components as those of other mammals (Befu, 2009). However, the morphological and histological features of the VNO in bears have not been comprehensively studied. The present study aimed to determine the properties and degree of development of the VNO in the brown bear (*Ursus arctos*).

Materials and Methods

Animals

Table 1 summarizes individual information about four captive bears (natural or accidental death) at Noboribetsu Bear Park (Noboribetsu, Hokkaido, Japan) and three wild bears killed for nuisance control in Hokkaido, Japan. The Animal Care and Use Committee of Obihiro University of Agriculture and Veterinary Medicine was notified of the experimental protocol (Notification No. 28-51) and the study proceeded according to Institutional Regulations on the Management and Operation of Animal Experiments.

Antibodies

The primary antibodies were rabbit polyclonal antibodies against olfactory marker protein (OMP) of human origin (sc-67219; Santa Cruz Biotech., Santa Cruz, CA, USA), human $G_{\alpha i2}$ (ab20392; Abcam, Cambridge, MA, USA) and rat $G_{\alpha o}$ (sc-387; Santa Cruz). The secondary antibody was biotinylated goat polyclonal antibody against rabbit IgG (BA-1000; Vector Lab., Burlingame, CA, USA).

Topographical procedures

The topographical features of the VNO were examined in two heads. Bones forming the left nasal side were sagittally sawed to generate a lateral view of the left VNO. The basal position of the nasal septum was sawed frontally in series, and the right VNO was observed using an SMZ1500 stereomicroscope (Nikon, Tokyo, Japan). The shape of the vomeronasal cartilage was established based on a series of frontal sections of the VNO.

Histological and conventional histochemical procedures

The VNO and the olfactory mucosa covering the ethmoidal conchae were fixed with Bouin's fluid and then embedded in paraffin using the standard procedure. Specimens were frontally cut into 5-μm-thick sections, deparaffinized and stained with hematoxylin-eosin, periodic acid-Schiff (PAS) or Alcian blue (AB) pH 2.5. Stained sections were assessed using a Microphot-FX microscope (Nikon) equipped with a Digital Sight DS-5M camera (Nikon).

Immunohistochemistry

Specimens were histochemically processed as described (Kondoh et al., 2017a).

Briefly, deparaffinized 5-μm-thick sections were incubated with 0.3% H_2O_2 in methanol, followed by 3% normal goat serum. The sections were incubated at 4°C overnight with a primary antibody (anti-OMP, 2.0 μg/mL; anti-$G_{\alpha i2}$, 2.5 μg/mL; anti-$G_{\alpha o}$, 2.0 μg/mL) followed by the secondary antibody (7.5 μg/mL) for 60 min. Thereafter, the sections were immersed in avidin-biotin-peroxidase complex (PK-6100; Vector) for 30 min and then visualized using 0.02% 3,3'-diaminobenzidine tetrahydrochloride in Tris-HCl buffer containing 0.006% H_2O_2.

Transmission electron microscopy (TEM)

The VNO was trimmed into small blocks and fixed in 0.1M phosphate buffer (pH 7.4) containing 3% glutaraldehyde for 60 min. After washing with phosphate buffer, post-fixed with 1% OsO_4 in phosphate buffer for 60 min, dehydrated and then embedded in epoxy resin. Ultrathin sections (80 nm thick) were cut using a diamond knife and examined using an HT7700 transmission electron microscope (Hitachi, Tokyo, Japan) without uranyl acetate and lead citrate staining.

Results

Topographical features of the VNO

Several nerves projected into the brain from the VNO, which was identified as a paired organ attached to the vomer bone that formed the basal anchorage of the nasal septum at the rostral half of the nasal region (Fig. 1A). The lumen of the VNO continued to the incisive duct that opened into the mouth (Fig. 1B). The VNO comprised of the cartilage and the soft tissue that contained the vomeronasal lumen (Fig. 1C). The

vomeronasal cartilage was attached to the nasal septum, and it covered the medial part of the soft tissue from the rostral to the caudal ends (Fig. 2A) and possessed a slit in the middle (Fig. 2A, G, H). The lateral part of the soft tissue was surrounded by dorsal and ventral protrusions of the cartilage at the rostral end (Fig. 2B) and middle (Fig. 2D-I) regions, respectively, and cross-section of the middle region revealed a J-shaped cartilage (Fig. 2D-I, L). The vomeronasal lumen was planiform at the rostral end (Fig. 2B, C), round at the rostral region (Fig. 2D, E), lunar at the middle region (Fig. 2F-I, L) and round at the caudal region (Fig. 2J) followed by a blind end (Fig. 2K).

Components of soft tissue of the VNO

The medial and lateral walls of the vomeronasal lumen were covered by the VNE and NSE, respectively, at the middle region of the longitudinal axis (Fig. 3A). The lumen of the middle position of the VNO partly invaginated toward the ventral region, and this invagination was covered by the NSE (Fig. 3B). Several large vomeronasal veins were located in the lateral and ventral parts of the lamina propria, although small vessels were scattered throughout the VNO (Fig. 3A). A few arteries were also located in the ventral lamina propria (Fig. 3A). Large vomeronasal branched glands were located at dorsal and lateral parts of the lamina propria (Fig. 3A), whereas only a few small glands were located in the medial lamina propria. A few small unbranched glands were also present just beneath the NSE (Fig. 3A). Nerve bundles were located in the medial and dorsal lamina propria (Fig. 3A).

Histological features of the VNE and NSE

Both the VNE and the NSE were pseudostratified in VNO. The VNE consisted of

supporting, sensory and basal cells (Fig. 4A). The sensory cells possessed round nuclei that were arranged in 1-2 cell layers at the middle region (Fig. 4A), and the cell bodies and dendrites were positive for anti-OMP(Fig. 5A). Cilia (Fig. 4A, 5E, F, G) and microvilli (Fig. 4A, 5E, F) extended from dendritic knobs. The dendritic knobs were positive for anti-$G_{\alpha i2}$ antibody (Fig. 5B) but apparently negative for anti-$G_{\alpha o}$ antibody (Fig. 5C), although anti-$G_{\alpha o}$ antibody widely reacted to cell components of the VNE including the supporting cells (Fig. 5C). No structures were stained in negative controls (Fig. 5D). The concentration of sensory cells (100-200 cells/ mm epi), the mean size of nuclei of sensory cells (14.6-22.8 μm^2) and the mean thickness of VNE (37.4-65.2 μm) varied according to individuals. These differences did not depend on the sex, but the concentration of sensory cells and the mean thickness of VNE seemed to decrease with the age. The supporting cells possessed oval nuclei which were arranged in 3-4 cells at the apical region, whereas the basal cells possessed irregular nuclei that were scattered at the basal region. On the other hand, nuclei of the sensory and supporting cells in the olfactory epithelium were arranged in 4-6 and 1-2 cell layers, respectively (Fig. 4B).

The NSE was mainly composed of columnar, goblet and basal cells as well as multicellular intraepithelial gland cells (Fig. 4C). The respiratory epithelium in the nasal cavity comprised only columnar, goblet and basal cells (Fig. 4D).

Properties of secretory cells of the vomeronasal glands, multicellular intraepithelial gland cells and goblet cells in the VNO

Vomeronasal glands were mainly located in the dorsal and lateral regions of the lamina propria (Fig. 3A, Fig. 6). Many goblet cells were found in the NSE covering the invagination of the ventral region, and tightly-packed multicellular intraepithelial gland

191 cells were located in the smaller branches of this invagination (Fig. 6). Several small acini
192 of the vomeronasal glands were also found around this invagination (Fig. 6G). The
193 structure of gland cells invagination in males seemed to spread a wide range rather than
194 that in females.

195 Round nuclei were located in the center of the cytoplasm within secretory cells of
196 the vomeronasal glands (Fig. 6D), whereas planiform nuclei of multicellular
197 intraepithelial gland cells were basally located in a relatively large amount of cytoplasm
198 (Fig. 6G). The vomeronasal glands were positive for PAS, but negative for AB staining
199 (Fig. 6E, F), whereas multicellular intraepithelial gland cells and goblet cells in the NSE
200 were positive for both (Fig. 6H, I). The free border of the VNE was covered by PAS- and
201 AB-positive mucosal fluid (Fig. 6K, L).

202 Histological features of the secretory cells in the VNO described above did not
203 differ among all seven animals examined in this study, and then we analyzed a represent
204 specimen by TEM. Observation using TEM showed that secretory cells in the
205 vomeronasal glands possessed small granules (diameter, 0.3–1 μm), some of which
206 contained several small high-density cores (Fig. 7A, B). On the other hand, multicellular
207 intraepithelial gland cells possessed large granules (diameter, 2–4 μm), most of which
208 contained a single large high-density core (Fig. 7C, D). Goblet cells possessed medium-
209 sized granules (diameter, 1–2 μm) without core structures (Fig. 7E, F).

210

211

212 **Discussion**

213 The VNO is distributed in most terrestrial mammals (Broom, 1900; Wysocki, 1979;
214 Halpern, 1987; Døving & Trotier, 1998; Brennan, 2001), although marine mammals

(Mackay-Sim et al., 1985; Oelschläger, 1989), many bats (Wible & Bhatnagar, 1996) and catarrhine primates (Bhatnagar & Meisami, 1998) lack this organ. The VNO of brown bears is located at the same nasal position as in many other mammals (Broom, 1900; Wysocki, 1979; Halpern, 1987; Døving & Trotier, 1998; Brennan, 2001). The orifice of the VNO differs according to species (Allison, 1953; Brennan, 2001). The vomeronasal duct of rodents (Taniguchi & Mochizuki, 1983; Mendoza, 1993), rabbits (Taniguchi & Mochizuki, 1983) and some bats (Cooper & Bhatnagar, 1976) opens directly into the nasal cavity, while the VNO of most carnivores (Adams & Wiekamp, 1984; Salazar et al., 1996), ungulates (Kratzing, 1971; Salazar et al., 2008, Lee et al., 2016) except horse (Salazar et al., 1997), platyrrhini primates (Smith et al., 2011) and marsupials (Poran, 1998; Schneider et al., 2008) in addition to other bats (Cooper & Bhatnagar, 1976) generally connects to the mouth via the incisive duct. As with other carnivores (Adams & Wiekamp, 1984; Salazar et al., 1996), the VNO of the bear opened to the mouth. Flehmen behavior is conspicuous in ungulates and carnivores to sample pheromones (Evans, 2003), and bears also seem to uptake pheromones into the VNO via the incisive duct opening to the oral cavity by this behavior (Stirling et al., 2016).

The soft tissue of the VNO in mammals is generally covered by cartilage, although that in rodents (Taniguchi & Mochizuki, 1983; Mendoza, 1993) is surrounded by bone capsule. The vomeronasal cartilage of ungulates (Salazar et al., 1995; Besoluk et al., 2001; Park et al., 2014; Kondoh et al., 2017) completely or roughly surrounds the soft tissue components (O-shaped cartilage), while that of platyrrhini primates (Smith et al., 2011) and marsupials (Poran, 1998; Schneider et al., 2008) does not entirely cover it (J-shaped capsule). In carnivores (Salazar et al., 1995) and bats (Cooper & Bhatnagar, 1976), the cartilage types (O- or J-shaped capsules) vary according to species. Among carnivores,

239 the VNO of cat and mink is encircled by the cartilage, while that of dog possesses the J-
240 shaped cartilage (Salazar et al., 1995). The whole image of the vomeronasal cartilage of
241 dog, cat and mink is also indicated by Salazar et al. (1995), and the shape of the cartilage
242 in the brown bear seems essentially similar to that in dog among carnivores.

243 The VNO of brown bears contains VNE, NSE, vomeronasal glands, vomeronasal
244 cartilage and vessels like that of Asiastic black bears (Befu, 2009), and histological
245 features of the brown bear VNE are largely similar to those of dog (Salazar et al., 2013).
246 Receptor cells expressing OMP, which is known as a neuronal marker of differentiated
247 receptor neurons of olfactory system (Weiler & Benali, 2005), in the brown bear VNO
248 possessed both cilia and microvilli, although these cells in mammals generally only have
249 microvilli (Miragall et al., 1979; Taniguchi & Mikami, 1985). The VNO of dog (Adams
250 & Wiekamp, 1984), but not cat (Salazar et al., 1996) has receptor cells with cilia and
251 microvilli, features that seem specific to the suborder Caniformia (Carnivora, Mammalia).
252 The cell types comprising the NSE in the brown bear were similar to those in other
253 mammals including dog and cat (Adams & Wiekamp, 1984; Salazar et al., 1996), except
254 for the multicellular intraepithelial glands that might secrete substances in the mucosal
255 fluid covering the vomeronasal lumen, like vomeronasal glands and goblet cells.

256 The vomeronasal receptors comprise the V1Rs coupled with $G_{\alpha i2}$ and V2Rs
257 coupled with $G_{\alpha o}$ (Dulac & Axel, 1995; Herrada & Dulac, 1997; Matsunami & Buck,
258 1997; Ryba & Tirindelli, 1997). The VNO of rodents, marsupials and monotremes
259 possesses both V1Rs-$G_{\alpha i2}$ and V2Rs-$G_{\alpha o}$ (Jia & Halpern, 1996; Shi & Zhang, 2007:
260 Brykczynska et al., 2013). On the other hand, the vomeronasal system exclusively
261 expresses V1Rs-$G_{\alpha i2}$ and degenerates functional V2Rs in most carnivores, ungulates and
262 platyrrhini primates (Takigami et al., 2000, 2004; Shi & Zhang, 2007; Young & Trask,

2007; Young et al., 2010; Salazar & Sánchez-Quinteiro, 2011; Hohenbrink et al., 2012; Salazar et al., 2013; Brykczynska et al., 2013; Dinka et al., 2016), while that of scaled reptiles exclusively possesses V2Rs-$G_{\alpha o}$ and degenerates functional V1Rs (Kondoh et al., 2013; Brykczynska et al., 2013). The present immunohistochemical findings showed that $G_{\alpha i2}$, but not $G_{\alpha o}$, is expressed in dendritic knobs of the vomeronasal sensory cells in the bear where receptors were located, indicating that the vomeronasal system mainly expresses V1R-$G_{\alpha i2}$ in bears as it does in other carnivores. As V1Rs bind to small and volatile molecules (Leinders-Zufall et al., 2000), bears might detect the pheromones in the air using the VNO, a notion that is supported by the fact that bears exhibit flehmen behavior (Gonzales et al., 2013).

The present histological, histochemical, and ultrastructual findings showed that the bear VNO possesses three types of secretory cells; PAS-positive and AB-negative secretory cells of vomeronasal glands, PAS-positive and AB-positive multicellular intraepithelial gland cells and goblet cells. The VNO of most mammals that have been investigated (Salazar et al., 1996, 1997; Roslinski et al., 2000; Ibrahim et al., 2013; Lee et al., 2016; Kondoh et al., 2017b) possesses either PAS-positive and AB-positive secretory cells of the vomeronasal glands (cow, sheep and giraffe) or PAS-positive and AB-negative secretory cells (cat, horse, lemur and vole), in addition to goblet cells. Wallaby possesses only PAS-positive and AB-negative glands, although a few cells do react to AB (Schneider et al., 2008). To our knowledge, however, multicellular intraepithelial gland cells, in addition to the invagination of the lumen, in the VNO were not detected in any mammals which have been reported. Therefore, multicellular intraepithelial gland cells which are mainly contained in the invagination of the ventral region seem unique features of the VNO of brown bears. These interspecies differences

indicate that properties of the mucosal fluid in the VNO vary, and are probably associated with pheromone reception (Khew-Goodall et al., 1991).

It is reported that the histological features of VNE are affected by sex (Segovia & Guillamon, 1993) and age (Wilson & Raisman, 1980) in rodents. In bears, the concentration of receptor cells and the mean thickness of VNE seem to depend on age. In addition, the structure of gland cells invagination in males seems more complex than that in females. However, more detailed research is necessary to clarify the age- and sex-dependent difference of the bear VNO.

In conclusion, our detailed morphological and histological examination revealed that the VNO of brown bear, especially the secretory system, is well-developed, suggesting that it is significant for information transmission in bears.

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Table 1. Topographic, histological, histochemical and transmission electron microscopy (TEM) investigations of seven brown bears

ID	Sex	Age† (y)	Topography	Histology and histochemistry				TEM	Source
				VNE	VNG	OE	RE		
A	Male	3	✓		✓	✓	✓		Nuisance control in Hokkaido, Japan.
B	Male	1	✓	✓	✓	✓	✓		Noboribetsu Bear Park (Noboribetsu, Hokkaido, Japan)
C	Female	> 30			✓				Noboribetsu Bear Park (Noboribetsu, Hokkaido, Japan)
D	Female	> 30		✓	✓				Noboribetsu Bear Park (Noboribetsu, Hokkaido, Japan)
F	Male	Immature		✓	✓	✓	✓	✓	Nuisance control in Hokkaido, Japan.
G	Female	Immature		✓	✓	✓			Nuisance control in Hokkaido, Japan.
I	Female	21		✓	✓				Noboribetsu Bear Park (Noboribetsu, Hokkaido, Japan)

†Ages of B, C, D and I determined from the Park records; that of A determined from aging of the cementum layer of teeth. Ages of F and G were not estimated, but their testis or ovary were immature.

OE, olfactory epithelium; RE, respiratory epithelium; VNE, vomeronasal epithelium; VNG, vomeronasal gland

Figure legends

Fig. 1. Topography of brown bear vomeronasal organ.

A Left lateral view of nasal region after removing the lateral-covering bones and rostral region of nasal septum. * Caudal part of the nasal septum. Vomeronasal organ is located at basal region of nasal septum (dashed circle) and projects several vomeronasal nerves (arrows) to brain. Abbreviations: dnc, dorsal nasal concha of left side; ec, ethmoidal concha of left side; vnc, ventral nasal concha of right side. **B** Left ventro-lateral view of anterior region of the palate. Vomeronasal organ (VNO) opens at incisive papillae (asterisk) via incisive duct (id; after sectioned). An insert shows the incisive duct before sectioning (dashed line). **C** Cartilage (dashed line) and soft tissue (asterisk) comprising VNO. Left side of panels shows rostral and upper dorsal areas. Bar = 10 mm.

Fig. 2. Morphological features of vomeronasal cartilage and lumen in brown bear.

A Schema of cartilage (lower) and corresponding lateral view of nasal region (upper). Left side of panel shows rostral and upper dorsal areas. **B-K** Series of illustrations showing frontal section of vomeronasal cartilage (filled drawing) and lumen (asterisks) based on stereomicroscopy findings. Dashed lines in A correspond to panels, which show sections from rostral panel B to caudal panel K. Abbreviations: b, bone; cns, cartilage of nasal septum; nc, nasal cavity. Left side of figures is lateral and upper dorsal. Bar (B) = 2 mm. **L** Stereomicroscopic image corresponds to panel (**G**).

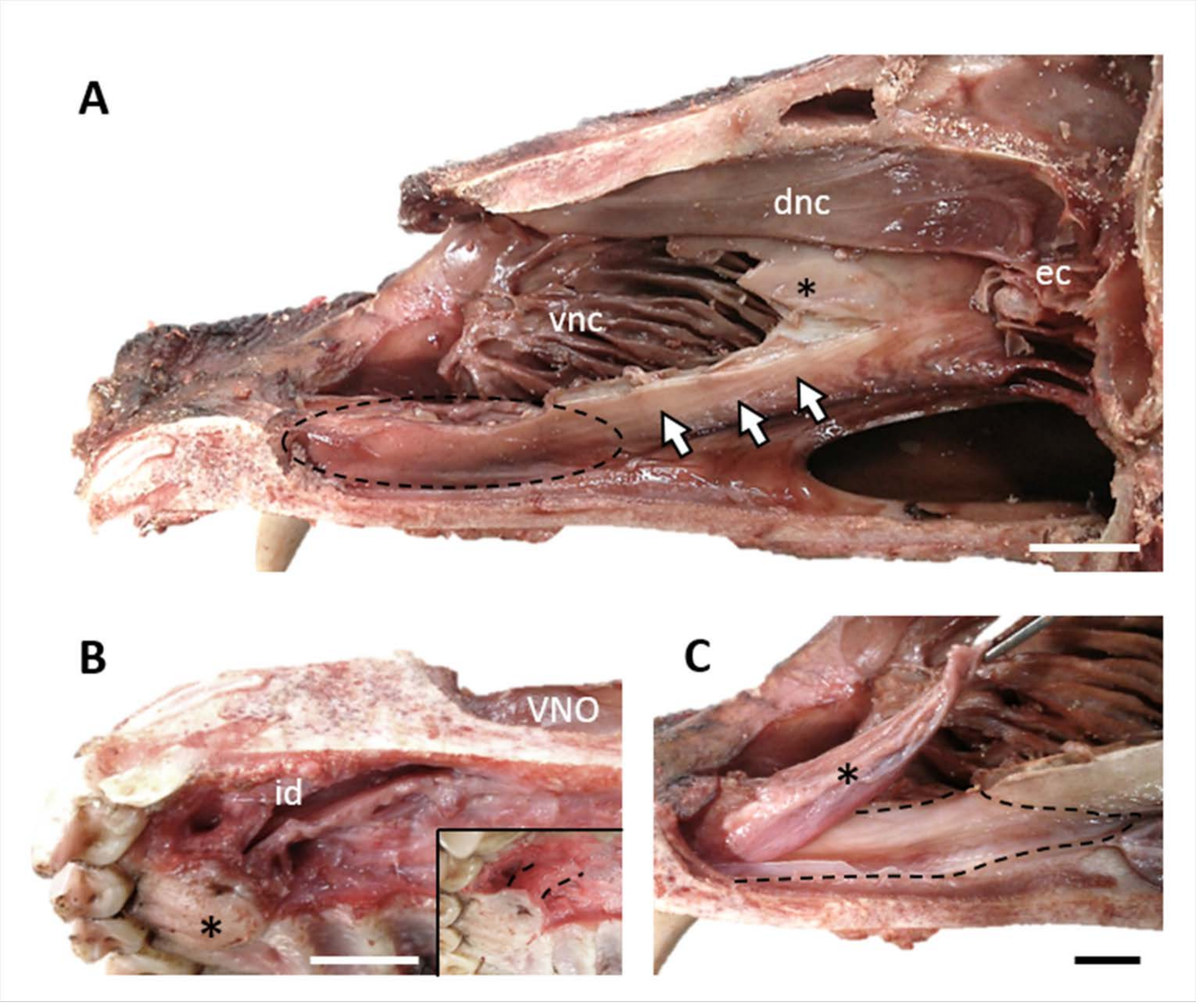
Fig. 3. Histological features of vomeronasal organ in brown bear.

A, B Frontal section of the middle region at a longitudinal axis. Panel **A** represents general shape of the vomeronasal lumen, and panel **B** shows the specific invagination structure

474 toward the ventral region. Arrow and arrowheads indicate duct and acini of vomeronasal
475 glands, respectively. Double arrows indicate unbranched glands immediately below
476 surface epithelium, and double arrowheads indicate opening part of the invagination
477 structure. Abbreviations: a, artery; b, bone; c, cartilage; NSE, non-sensory epithelium;
478 RE, respiratory epithelium in the nasal cavity; VNE, vomeronasal sensory epithelium; v,
479 vein; *, Nerve bundles. Left side of panel is lateral and upper, dorsal. Bars = 1 mm.
480
481 **Fig. 4.** Histological features of the epithelia in the vomeronasal organ and nasal cavity in
482 brown bear.
483 **A** Histological structures of the vomeronasal sensory epithelium (VNE). Arrow and
484 arrowheads indicate cilia and microvilli, respectively. Abbreviations: BC, nuclei layer of
485 the basal cells; RC, nuclei layer of the receptor cells; SC, nuclei layer of the supporting
486 cells (**A** and **B**). **B** Histological structures of olfactory epithelium (OE). **C** Histological
487 structures of the non-sensory epithelium (NSE) in the vomeronasal organ. *Multicellular
488 intraepithelial glands. **D** Histological structures of respiratory epithelium (RE) in the nasal
489 cavity. Bars = 20 μm.
490
491 **Fig. 5.** Immunohistochemical and ultrastructural features of vomeronasal sensory
492 epithelium in brown bear.
493 Immunoreactivity against anti-OMP (**A**), -$G_{\alpha i2}$ (**B**) and -$G_{\alpha o}$ (**C**) antibodies and negative
494 control without antibodies (**D**). Arrowheads indicate dendritic knobs positive for anti-
495 OMP and -$G_{\alpha i2}$ but negative for anti-$G_{\alpha o}$. **B-C** Insert figures show the dendritic knobs at
496 high magnification. BC, nuclei layer of basal cells; RC, nuclei layer of the receptor cells;
497 SC, nuclei layer of the supporting cells (**A-D**). Vertical (**E, F**) and horizontal (**G**) images

498 of dendritic ultrastructure of the receptor cell (RC). Arrows and arrowheads indicate cilia
499 and microvilli, respectively. SC, supporting cell. K, dendritic knobs. Mt, mitochondria.
500 *Basal bodies. Bars = 20 (**A-D**), 1(**E and F**) and 0.5(**G**) μm.
501
502 **Fig. 6.** Histological and histochemical features of three types of secretory cells in the
503 vomeronasal organ of brown bear.
504 Hematoxylin-eosin (**A, D, G, J**), periodic acid-Schiff (PAS) (**B, E, H, K**) and Alcian blue
505 (pH 2.5, AB) (**C, F, I, L**) staining. **A-C** Locations of the vomeronasal glands (VNG) and
506 multicellular intraepithelial gland cells (arrowheads). Many multicellular intraepithelial
507 gland cells are located in the invagination of the ventral region (Iv). Areas surrounded by
508 dashed and solid boxes correspond to panels **D-F** and **G-I**, respectively, at higher
509 magnification. Abbreviations: NSE, non-sensory epithelium; VNE, vomeronasal sensory
510 epithelium. Left side of panels shows lateral and upper dorsal regions. **D-F** Vomeronasal
511 glands positive for PAS but negative for AB at dorsal and lateral regions of vomeronasal
512 organ. **G-I** Multicellular intraepithelial gland cells (dashed circles) and goblet cells
513 (arrowheads) positive for PAS and AB which are located in the invagination of the ventral
514 region of the vomeronasal organ. Several acini of the vomeronasal glands (asterisks) are
515 also found. **J-L** Mucosal fluid covering vomeronasal sensory epithelium is positive for
516 PAS and AB. Bars = 250 (**A**), 50 (**D, G, J**) μm.
517
518 **Fig. 7.** Ultrastructural features of three types of secretory cells in vomeronasal organ of
519 brown bear.
520 **A** Secretory cells of the vomeronasal glands. N, nucleus. **B** High magnification image of
521 panel **A**. Black dashed lines indicate secretory granules, and white dashed lines indicate

522 tiny high-density cores in granules. **C,** Multicellular gland cells. N, nucleus. **D** High
523 magnification image of panel **C**. Black dashed lines indicate secretory granules, and white
524 dashed lines indicate large high-density cores within granules. **E** Goblet cell (GC) in non-
525 sensory epithelium. CC, columnar cell. **F** High magnification image of panel **E**. Dashed
526 lines indicate secretory granules. Bars = 5 (**A, C, E**) and 1 (**B, D, F**) μm.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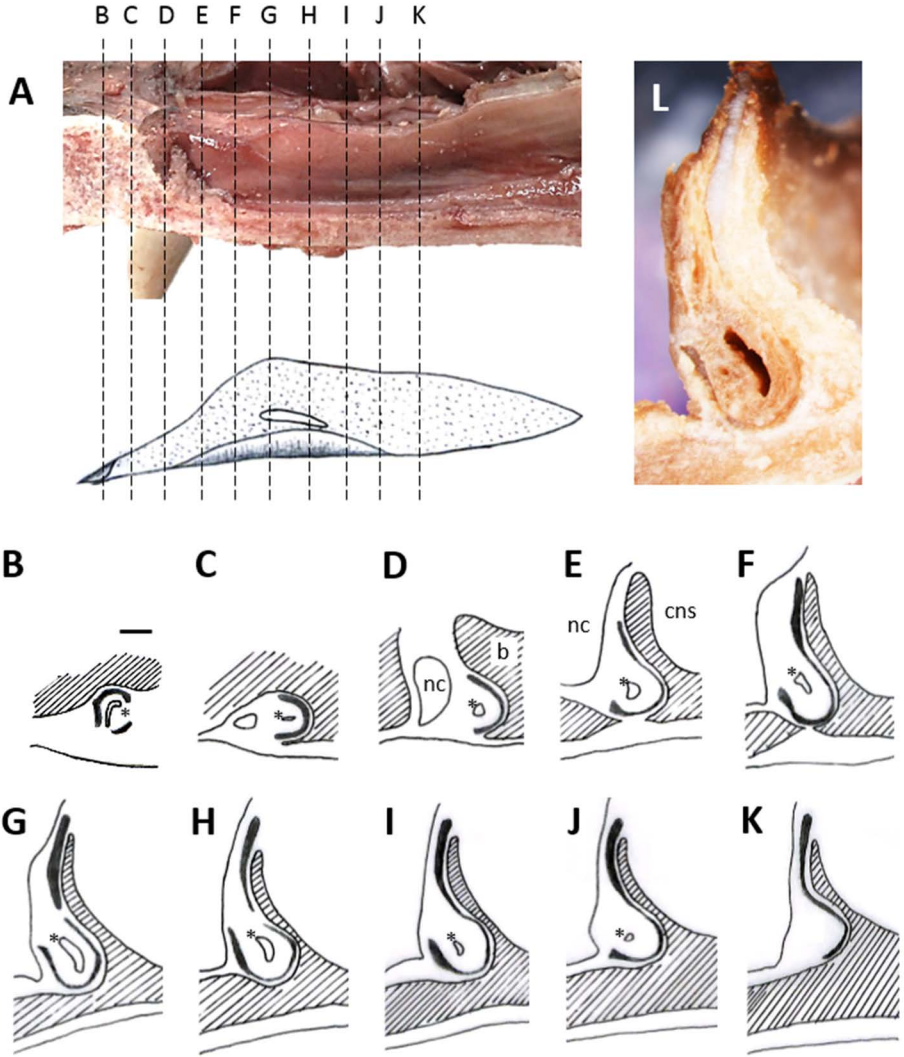
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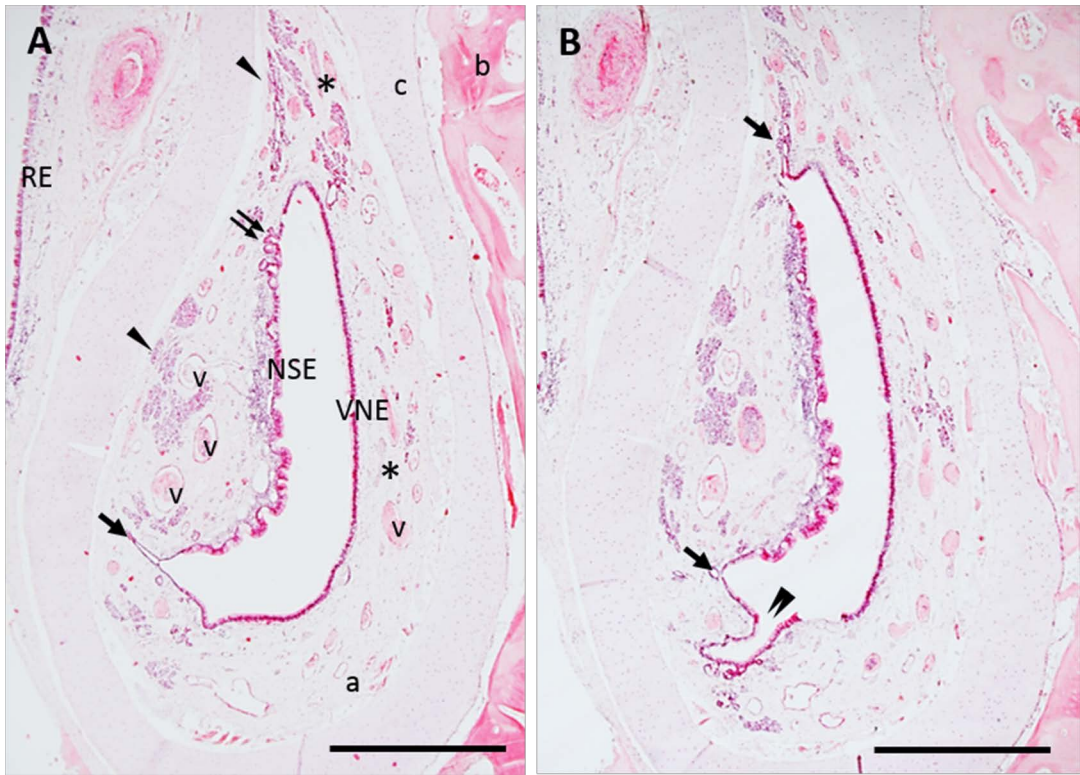
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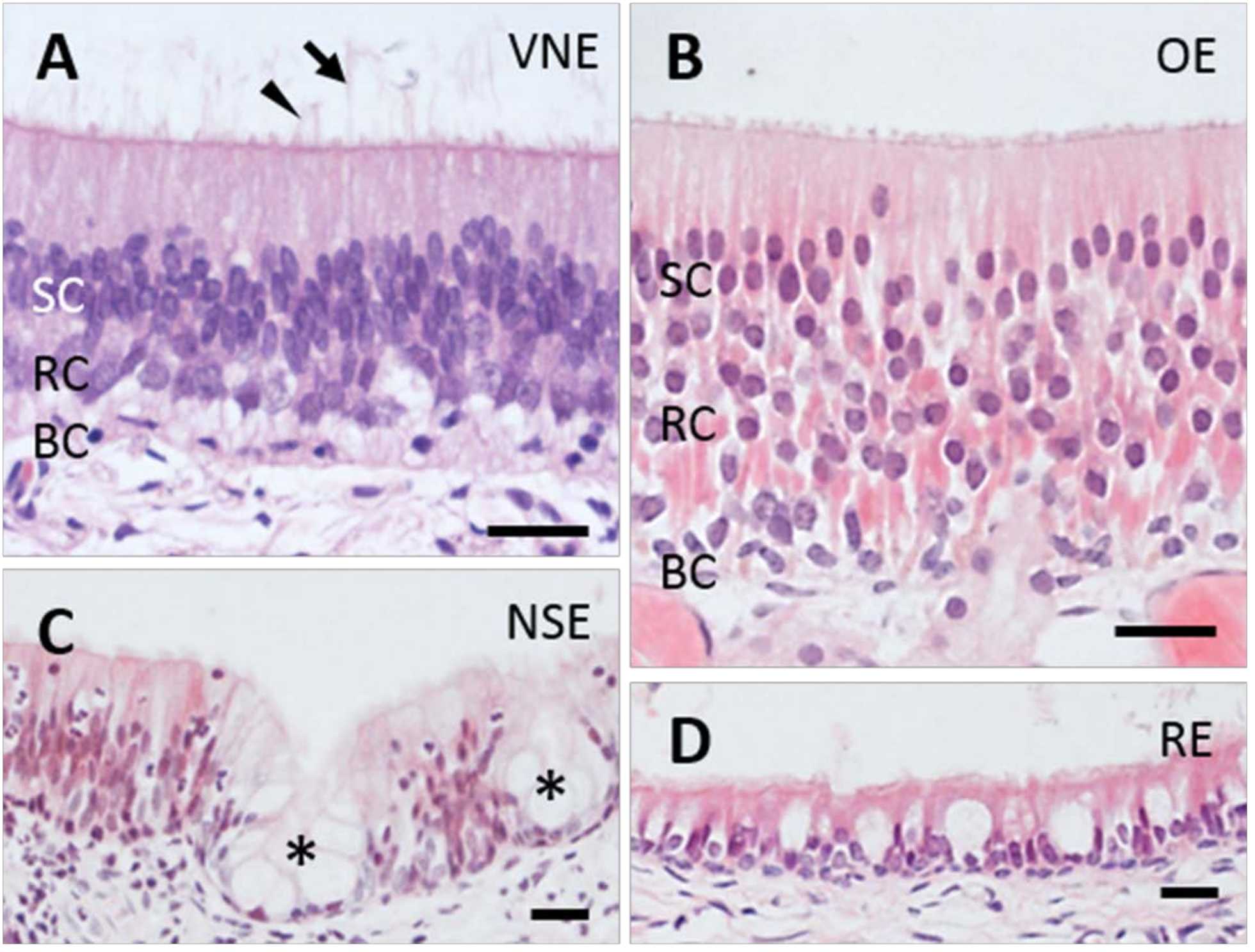
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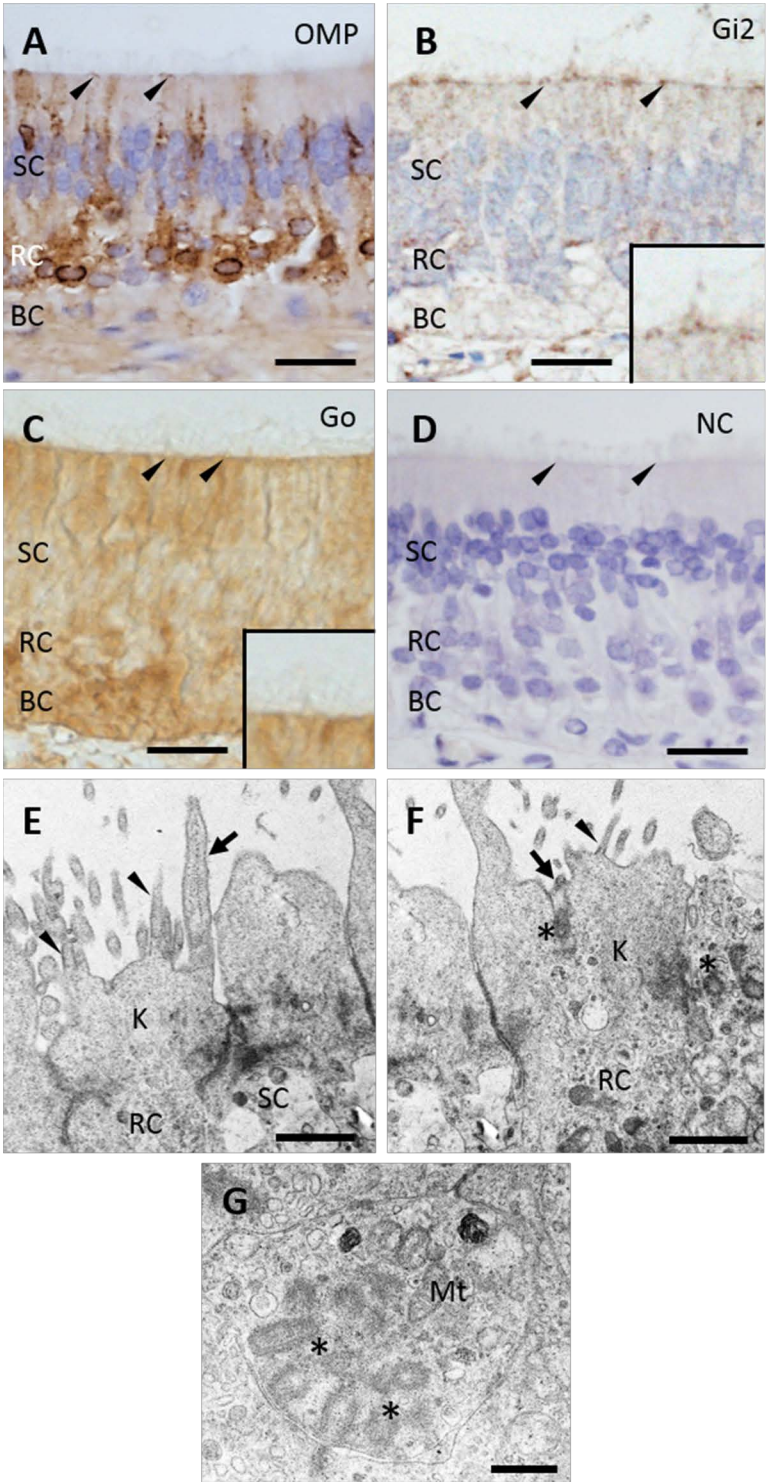
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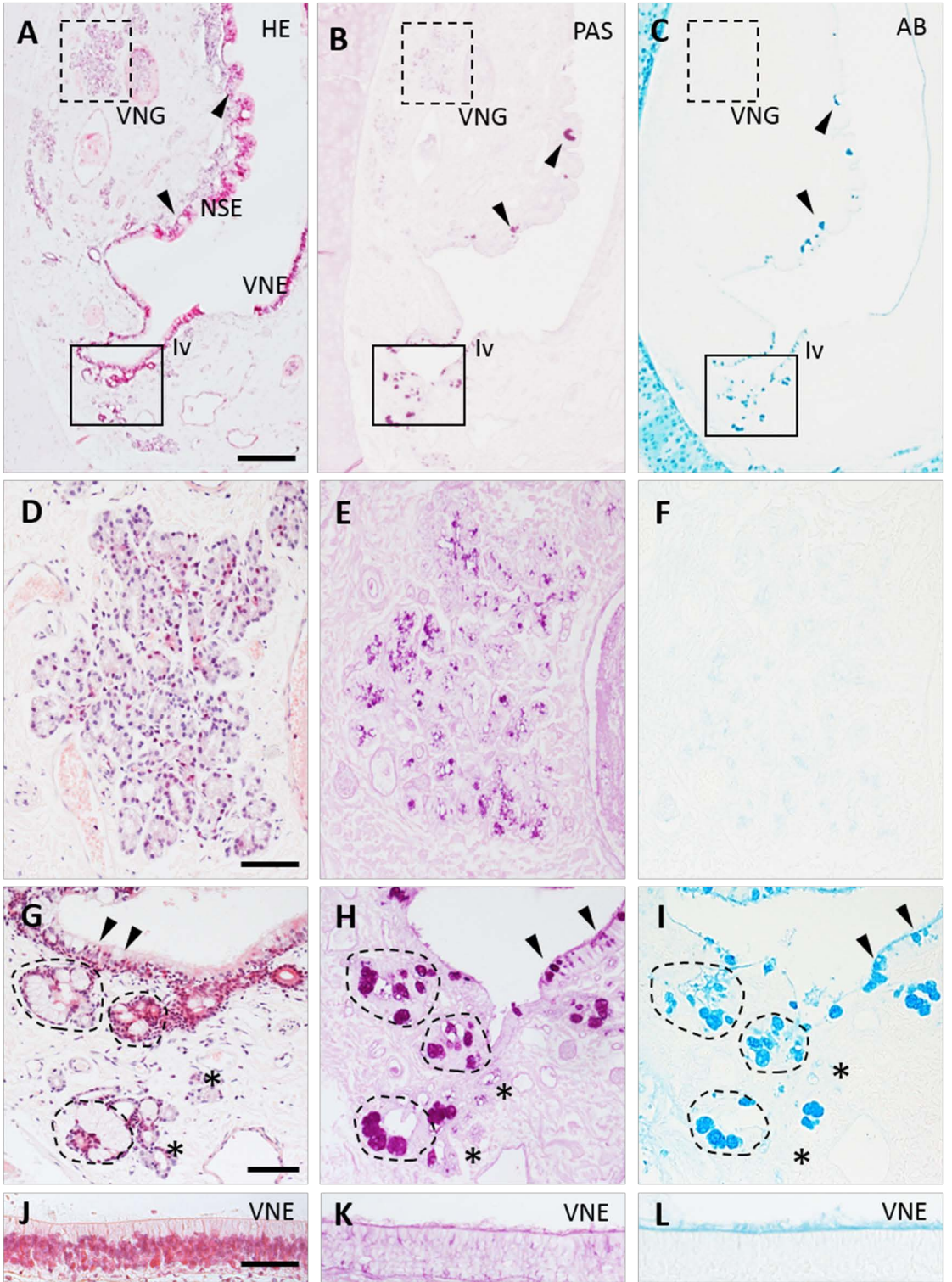
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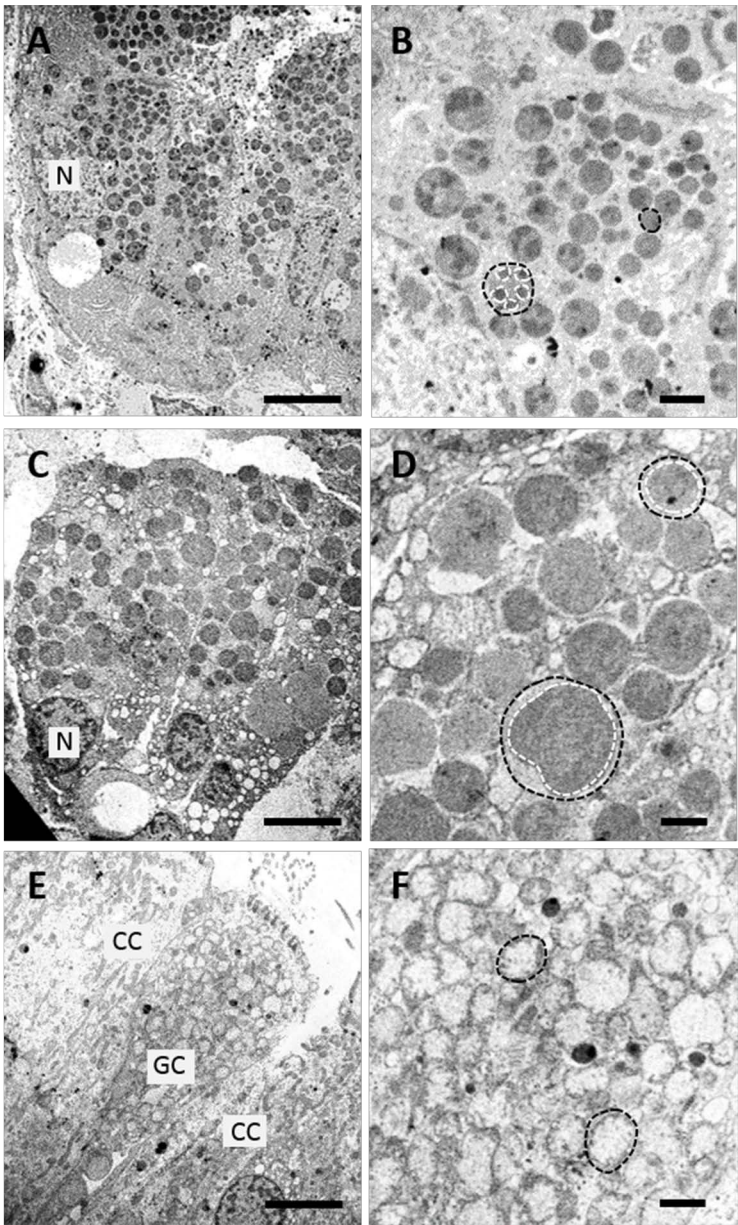
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