Is the Mole Rat Vomeronasal Organ Functional?

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ABSTRACT

The colonial naked mole rat *Heterocephalus glaber* is a subterranean, eusocial rodent. The *H. glaber* vomeronasal organ neuroepithelium (VNE) displays little postnatal growth. However, the VNE remains neuronal in contrast to some mammals that possess nonfunctional vomeronasal organ remnants, for example, catarrhine primates and some bats. Here, we describe the vomeronasal organ (VNO) microanatomy in the naked mole rat and we make preliminary observations to determine if *H. glaber* shares its minimal postnatal VNE growth with other African mole rats. We also determine the immunoreactivity to the mitotic marker Ki67, growth-associated protein 43 (GAP43), and olfactory marker protein (OMP) in six adult and three subadult *H. glaber* individuals. VNE volume measurements on a small sample of *Cryptomys hottentotus* and *Fukomys damarensis* indicate that the VNE of those African mole rat species are also likely to be growth-deficient. Ki67(+) cells show that the sensory epithelium is mitotically active. GAP43 labelling indicates neurogenesis and OMP(+) cells are present though less numerous compared to GAP43(+) cells. In this respect, the VNO of *H. glaber* does not appear vestigial. The African mole rat VNE may be unusually variable, perhaps reflecting reduced selection pressure on the vomeronasal system. If so, African mole rats may provide a useful genetic model for understanding the morphological variability observed in the mammalian VNO. Anat Rec, 00:000–000, 2019. © 2019 Wiley Periodicals, Inc.

Key words: adaptation; evolutionary; chemosensation; vestigial

The naked mole rat *Heterocephalus glaber*is a subterranean eusocial rodent that lives in colonies of up to 300 individuals and is indigenous to the horn of Africa (Brett, 1991). Subterranean species exhibit reduced visual acuity compared to surface species (Hetling, et al., 2005) but other senses are enhanced. These include the tactile

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sense (Crish et al., 2003; Catania, 2005) and chemosensory capacity in tracking food odorants (Heth et al., 2002; Lange et al., 2005) and, as in other rodents, scent marking of common nesting and toilet loci (Jarvis and Sherman, 2002). In particular, cues determining sexual activity in other rodents are mediated via the vomeronasal organ (VNO) (reviewed in Halpern, 1987 and more recently in Silva and Antunes, 2017). In naked mole rat colonies, the queen and a few males are active sexually but the remaining individuals are held in a sexually immature state. These individuals care for the young, excavate the burrow, and forage for the colony (Jarvis, 1981). This suppression is mediated by behavioral cues from the queen rather than by urinary signals detected by the VNO (Faulkes and Abbott, 1993; Smith et al., 1997a). That difference makes the naked mole rat apparently unique among rodents at least in regulating sexual development and behavior. The question therefore arises: is the naked mole rat VNO functional? That is, does the mole rat VNO mediate pheromonal cues that evoke behavioral or physiological changes? A general approach to the question of function is to compare anatomical structures and protein expression patterns present in the mole rat VNO with those of species known to possess a functional VNO.

Previously, Smith et al. (2007) measured the volume of the vomeronasal organ neuroepithelium (VNE) and, in contrast to rats, mice, and voles, found that the postnatal naked mole rat VNE does not increase in absolute volume. Further, the *H. glaber* VNO neuroepithelial component is 9.5-fold to 45-fold smaller than that of the mouse, rat, or vole. Conversely, VNE volumes of postnatal voles and rats increase by 5.5-fold to 8-fold, respectively (Weiler et al., 1999; reviewed in Smith et al., 2007). Limited VNE growth has not been linked directly to behavioral or ecological variables and it is not understood whether reduced growth indicates a functional reduction. Some bat and primate species with nonfunctioning vomeronasal systems lack key structures such as the VNO itself (Bhatnagar and Meisami, 1998; Smith et al., 2001; Bhatnagar and Smith, 2006). Other bats and primates retain VNO vestiges with supporting elements such as a cartilaginous capsule but lack connection to the brain (Bhatnagar and Meisami, 1998; Smith et al., 2001). In contrast, several rodent species possess a highly developed VNO system that includes a neuroepithelium, vomeronasal nerves, glands, venous sinuses, a surrounding capsule, and connections to the accessory olfactory bulb (Wysocki, 1979; Oikawa et al., 1994; Garrosa et al., 1998; Roslinski et al., 2000; Villamayor et al., 2018). These traits, common to the rodents studied to date, are presumably under strong stabilizing selection. By analogy, an overriding criterion of VNO function requires the presence of similar structures in *H. glaber* and other African mole rats.

Herein, we asked if *H. glaber* (family Heterocephalidae) is unique in postnatal VNE growth trajectory. For a preliminary determination, we compared volume, cross-sectional area, and VNE length of one neonate and one adult of two fossorial mole rat species of the family Bathyergidae: *Cryptomys hottentotus* and *Fukomys damarensis*. We compared those data with observations made from a sample of 15 *H. glaber* individuals and we describe the anatomy of the *H. glaber* VNO. We also performed a preliminary immunohistochemical analysis of VNE development from P1, subadult, and adult *H. glaber* individuals. Our previous report demonstrated that the *H. glaber* VNE remains neuronal throughout postnatal life (Smith et al., 2007) but, in order to be functional, VNE must also remain neurogenic across all life stages. Therefore, our immunohistochemical study included labeling with Ki67, GAP43, and OMP, which are markers of mitosis, neurogenesis, and olfactory sensory neuron maturation, respectively.

MATERIALS AND METHODS

We studied a sample of seven adults and seven subadult *H. glaber* individuals of which twelve had been analyzed histomorphometrically (Smith et al., 2007). One two-week-old and one adult specimen were newly prepared for this study. Subadults ranged from neonate (P1) to weaning age (P28). We also examined one adult and one newborn of the mole rat species *C. hottentotus* and *F. damarensis*. All specimens were acquired from breeding colonies maintained in the laboratory of TJP at the University of Illinois at Chicago. All procedures were approved by the IACUC at UIC. Infant (P0 to P14) mole rats were submerged in fixative after euthanasia. Adults were perfused through the heart under deep anesthesia with 0.9% saline for 15 sec followed by 10% formalin for 20 min and then decapitated. The heads were stored in formalin. One adult rat (*Rattus norvegicus*) was prepared in the same fashion and the procedure was approved by the IACUC at Auburn University.

Tissues were decalcified in formic acid, embedded in paraffin, sectioned at 10 μm, and mounted on glass slides. Every fifth section was mounted and stained using hematoxylin and eosin or Gomori trichrome. Intervening sections were archived for immunohistochemistry (IHC). We examined the vomeronasal complex structures which were VNE, receptor-free epithelium, vomeronasal nerves, glands, venous sinuses, and the surrounding capsule, that is, the vomeronasal cartilage and/or bony extensions from the palate. All specimens were observed at magnifications ranging from X25 to X630 using a Leica DMLB compound photomicroscope, with an Axiocam MRc 5 Firewire camera attached to the microscope. Across age, we recorded the microanatomical characteristics of these structures.

In the six newly prepared specimens (see above), the VNE was measured in length as described ion Smith et al. (2007). Each section containing the VNO was digitally photographed. The distance between the first and last sections containing VNNE was measured in μm by multiplying the number of sections by the known section thickness. ImageJ software (NIH) was used to measure VNE volumes. This was accomplished using micrographs scaled to a digital micrograph of a stage micrometer at the same magnification. The perimeter of the VNE was traced in the micrograph of each section, and the cross-sectional area was obtained in ImageJ. Each cross-sectional area was then multiplied by the distance between adjacent sections, resulting in segmental volumes. Segmental volumes were summed for total VNE volume. The total volume of the right VNE was calculated for each specimen; the left side was used in cases where the right VNE was damaged. Lastly, cross-sectional VNE areas for the sections from the 25th to 75th percentile were averaged to compute a mean value for each specimen. These data are discussed in terms of absolute VNE size rather than as ratios or residuals for two reasons. First, Smith and Bhatnagar (2004) argue that in special sense organs it is absolute rather than

proportional size that most likely reflects olfactory sensitivity. Second, in our previous work we found the VNE nuclear density to be similar in subadult and adult *H. glaber* (Smith et al., 2007), suggesting volumetric measurements correspond to similar per-unit sensory neuron numbers.

Of the 14 *H. glaber*, six adults and 3 subadults were used for immunohistochemistry. The archived material used from each specimen included sections that contained the VNO but also at least one section with olfactory neuroepithelium; the latter was used as a positive control. Sections used for immunohistochemistry were deparaffinized in Hemo-De, hydrated, equilibrated in PBS (pH 7.4), and blocked with 5% normal serum and 2.5% BSA in PBS. In the case of Ki67 assays, hydrated tissues were placed in 10 mM sodium citrate and boiled for 20 min. Afterward, the beaker containing the slides was cooled to room temperature (25°C) on the lab bench, and the slides were equilibrated in PBS. OMP (Wako Chemicals USA, Inc) (1:20000 or 1:22000), Ki67 (Thermoscientific) (1:2000), or GAP43 (Novus Biologicals) (1:24000) in blocker was applied and the sections were incubated at room temperature overnight. The following day, sections were incubated sequentially in 2-degree angle antibody for 1 hr and ABC reagent for 30 min, labeled with DAB, mounted, and examined with a Nikon Eclipse 600 microscope under bright field optics. One section in each assay received no primary antibody to serve as a negative control. Images were made with a Q-Imaging Retiga 2000R digital camera and Nikon Elements BR 2.3 Software.

The reactivity of the VNE to the primary antibodies was assessed by a comparison to the degree of reactivity in the olfactory neuroepithelium. Due to our limited sample size, the number of cells was estimated qualitatively as follows: nonreactive, if no reactive cells were observed; sparse reactivity, if only few neurons were reactive; moderate reactivity, if numerous cells were reactive, but fewer than in the positive control; strong reactivity, if the number of reactive neurons was similar to those in the positive control.

RESULTS

Descriptive Findings

Cryptomys hottentotus. Neonate (Figs. 1A and 2A): The vomeronasal cartilage (VNC) is surrounded by an extension from the maxilla at about 25% of VNO length. Rostral to this, the VNC is uniform in matrix characteristics. Where bone is adjacent to the VNC, the cartilage on the lateral side has a degenerated appearance. Glands appear minute with few acini and only small veins are present.

The VNE has multiple rows sensory neurons and appears thinner than in the newborn. The receptor-free epithelium is ciliated.

Adult (Figs. 1C and 2D): The VNC has bony reinforcement on its lateral sides along its entire length. At mid-length, the cartilage is reduced to a vertical strut and is absent laterally. Large glands and venous sinuses are within the capsule.

The VNE has multiple rows of sensory neurons and is thinner in absolute thickness than in the newborn. The receptor-free epithelium is ciliated.

Fukomys damarensis. Neonate (Figs. 1B, 2B, and 3A): The VNC is surrounded by an extension of the maxilla at the 20th percentile of total VNO length. The VNC is uniform in matrix characteristics only near its rostral opening. Where bone is adjacent to the VNC, the cartilage on the lateral side has a degenerated appearance. By the mid-rostrocaudal level, the VNC is fragmented in cross-section. A limited amount of glandular tissue is present in the capsule. The blood vessels (sinuses lateral to the VNO) are small in the rostral half of the VNO. Caudally, venous sinuses become progressively larger.

The VNE has multiple rows of sensory neurons. The receptor-free epithelium appears ciliated, but the preservation at the apical surface is imperfect (Fig. 3A).

Adult (Figs. 1D, 2E, and 3C): The VNC is J-shaped and mostly lacks bone lateral to it for the first third of rostrocaudal length. In the middle region, perichondrial bone is seen on lateral side of VNC and caudally the lateral VNO appears degenerated; in the caudal one-third it is no more than a vertical cartilaginous bar. Large venous sinuses occur lateral to the VNO and those sinuses encroach dorsally and ventrally at some cross-sectional levels. Glands in the adult nest mainly dorsal and partially medial to the VNO in cross-section.

The VNE has multiple rows of sensory neurons and is slightly thinner in absolute thickness than in the newborn. The receptor-free epithelium appears ciliated but this is not certain because of the preservation (Fig. 3C).

H. glaber. In the neonate individual, perichondrial bone lines both sides of the lateral limb of the VNC beginning near the rostrocaudal mid-point. Caudally, the lateral side of the VNC becomes fragmented with scattered vascular foci. As with the other mole rat species, the VNO fills the cross-sectional space within the VN capsule except for a thin rim of lamina propria and a small lateral venous sinus (Fig. 3B). In the P7 infant, the VNC is cartilaginous and J-shaped for roughly the rostral two-third of its length. The lateral limb of the "J" or "U" of the VNC ends abruptly at 2/3 of the rostrocaudal VNO length; instead, perichondrial bone is seen in place of the lateral limb. In adult naked mole rats, the extent of bone that contributes to the VN capsule varies. Between 33% and 53% of the capsule surrounding the VNO includes perichondrial bone. Perichondrial bone also covers the VN cartilage of the medial wall in some specimens. To some extent, the perichondrial ossification may be age-related since several of the oldest specimens lacked any remnants of cartilage in the caudal-most region.

The venous sinuses are far larger in the P14 infant than in the neonate. For the caudal-most, 20%–25% of VNO length, the VN capsule is composed entirely of bone and the venous sinuses have a greater surface area than the VNO itself. At P14, the degree of ossification of the VNC varies, but again is seen more in the caudal portion of the VNO length. Glands are better developed and are now seen both ventral and dorsal to the VNO.

The receptor-free epithelium is ciliated in newborns and adults. In some specimens, microvilli are well-preserved and visible on the VNE surface (Fig. 3D).

Quantitative Findings

The VNE volume of the adult *Cryptomys* specimen is 84% that of the neonate's VNE volume but the adult's VNE is 50% longer than that of the neonate. The

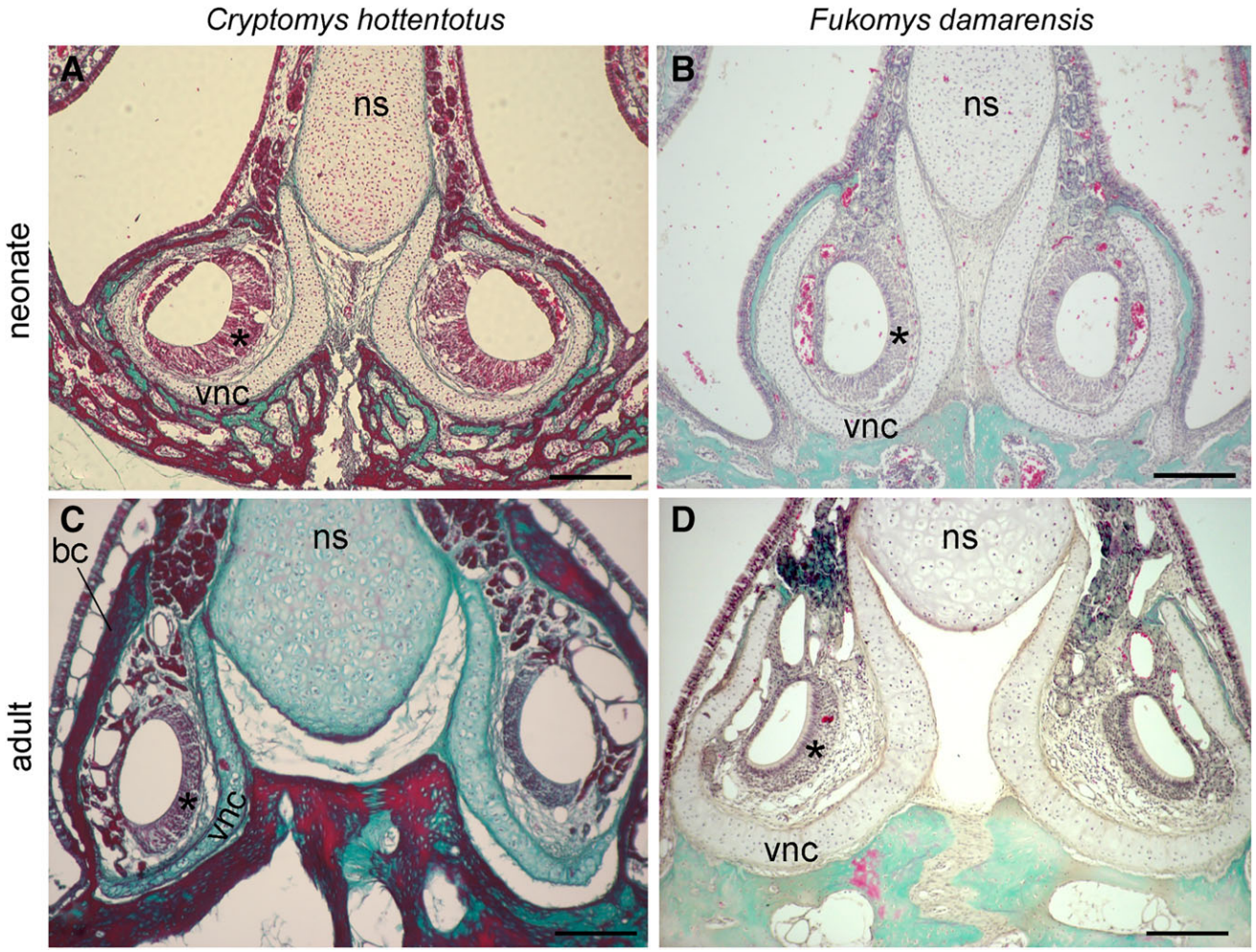

Fig. 1. The vomeronasal complex in newborn (**A**,**B**) and adult mole rats (**C**,**D**). All images are shown at identical magnifications. Note that in both species, the nasal septum (ns) is broader-based in adults, emphasizing differential growth compared to the vomeronasal complex. The vomeronasal cartilage (VNC) is taller and more ossified (bc, bony capsule) in the adult Hottentot mole rat (*C. hottentotus*), (C) and is more robust in the adult Damaraland mole rat (*F. damarensis*), (D) compared to newborns. In contrast, the vomeronasal organ and its neuroepithelium (*) are actually smaller in cross-section (at approximate mid-point along rostral-caudal axis) in adults compared to newborns. Scale bars, 200 μm.

apparent incongruity is explained by a decrease in cross-sectional area. The adult VNE cross-sectional area is little more than half as large as the neonate (Table 1).

The neonatal *Fukomys* VNE volume is 72% that of the adult although the adult VNE is 45% longer compared to the neonate. Again, the difference in VNE volume is accounted for by the difference in cross-sectional area, which is smaller in the adult (Table 1).

The adult *H. glaber* specimen's VNE average volume is 6% greater than that of the neonate. The adult VNE is 46% longer compared to the neonate but the adult VNE is 64% of the neonate's average cross-sectional area. The P7 individual's VNE is considerably smaller than the P1 VNE. If the two youngest infant measurements are combined, the comparison is as follows: average adult VNE volume is 22% greater; average length is more than twice greater in adults; average adult cross-sectional area is 85% that of the two infants.

Immunohistochemical Findings

Ki67. Two subadult individuals and one adult presented with reactive cells in the VNE (Table 2). In all cases, reactive cells were scattered and less densely distributed compared to those in the MOE (Fig. 4A). Ki67(+) cells were greatest in number in the newborn, even presenting within the receptor-free epithelium (Fig. 4B). A P7 infant presented similarly. A P28 specimen had sparsely distributed Ki67(+) cells within the VNE (Fig. 4C) and several Ki67(+) cells occur in an adult male (Fig. 4D). Two other adults were negative but artefactual folding and loss of most sections during antigen retrieval render the negative results unverifiable at present.

Growth-associated protein 43. Except in one case of artefactual damage, *H. glaber* tissues were Gap43(+) in the VNE and surrounding lamina propria and the degree of reactivity is similar to that of the

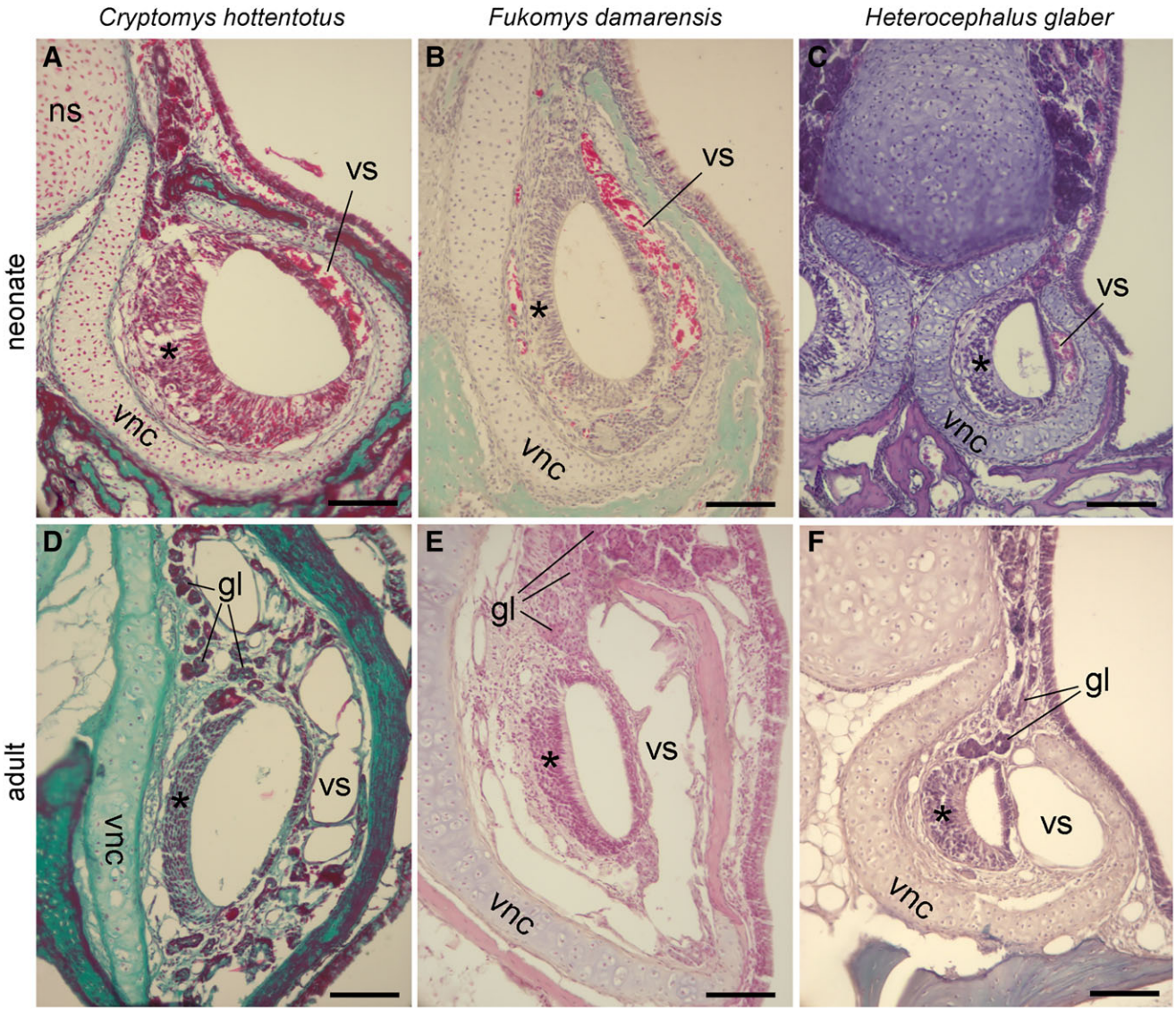

Fig. 2. Higher magnification view of the left vomeronasal organ (near mid-level rostrocaudally) and surrounding structures in three species of mole rats. (**A–C**) newborns; (**D–F**) adults. Note the cross-sectional area of the VNO appears similar or even smaller in adults compared to newborns; the vomeronasal neuroepithelium (*) is likewise appears similar or smaller in cross-sectional area in adults than neonates (also see Table 1). In the adults, the lamina propria surrounding the VNO is packed with more venous sinuses (*vs*) and glands compared to the newborns. Ns, nasal septum; VNC, vomeronasal cartilage. Scale bars, 100 μm.

internal control MOE (Fig. 5A). Reactivity within the VNE itself is weak in the P1 individual (Fig. 5B) but is prominent in older, subadult, and adult individuals (Fig. 5C). GAP43 reactivity is strongest in the transitional zone, the interface between the sensory and respiratory epithelium (Fig. 5D).

Olfactory marker protein. For comparison, rat (*Rattus norvegicus*) MOE (Fig. 6A) is strongly OMP(+) but label is less prevalent in the VNE (Fig. 6B). Both MOE and VNO nerve fascicles are strongly OMP (+). The *H. glaber* MOE is likewise more OMP-reactive than the VNE in all specimens (Figs. 6C,D and 7). Numerous OMP (+) nerve fascicles are present in the main olfactory lamina propria (Figs. 6C and 7A) but reactive vomeronasal nerve fascicles are fewer (Fig. 7D). All subadults and adults examined displayed intense OMP label in the MOE and nerve fascicles.

In *H. glaber,* the disparity between the MOE and VNE in OMP reactivity is greatest in the P1 specimen in which the former has reactivity within the epithelium and lamina propria, while the VNE is OMP(−) (Fig. 7A,C). Two adult females, one a breeding female, had OMP(+) vomeronasal sensory neurons (VSNs) evenly scattered throughout the VNE (Fig. 6D) but in all other adults the VNE has only sparse OMP(+) VSNs (Fig. 7B,D).

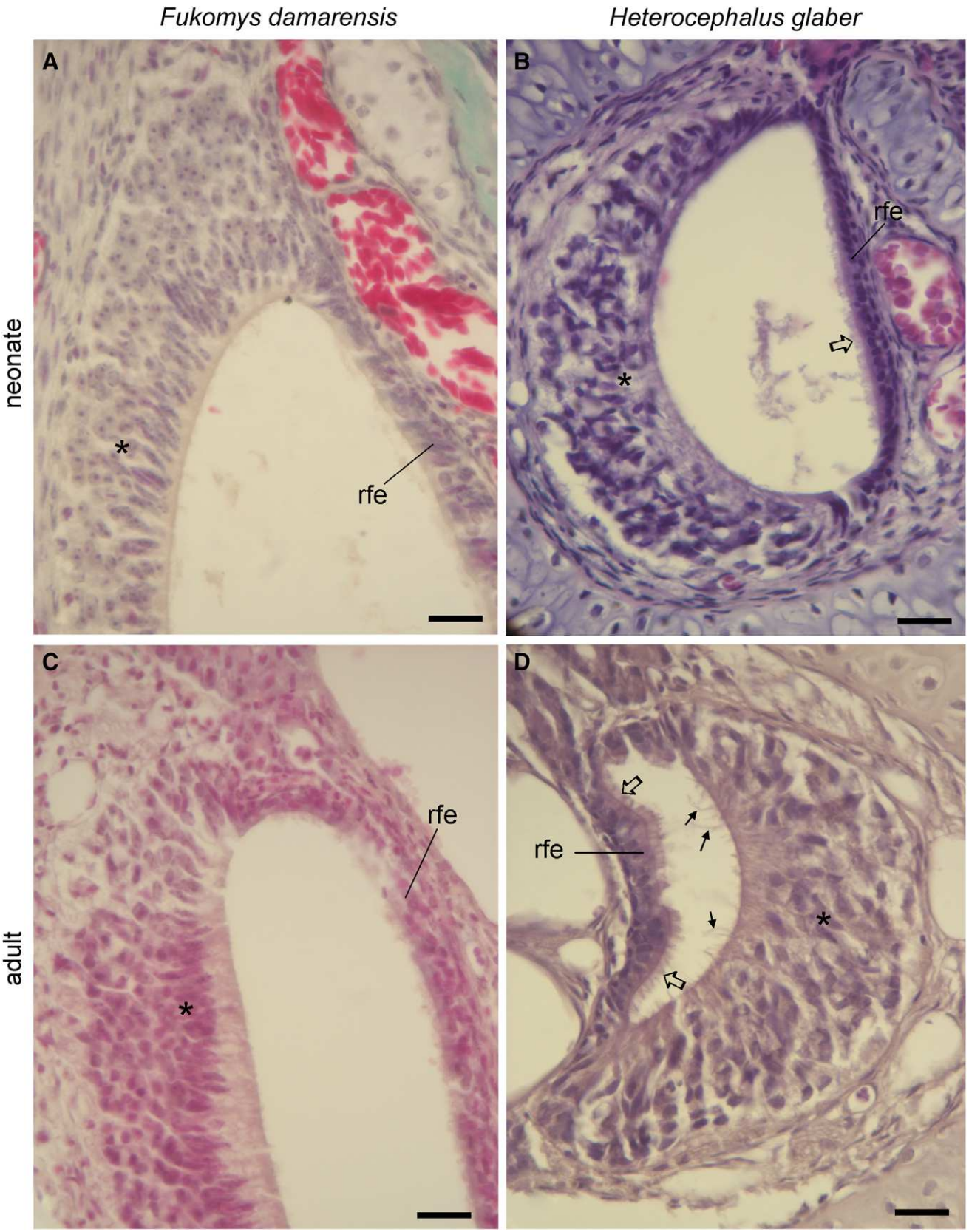

Fig. 3. Legend on next page.

TABLE 1. Measurements of the vomeronasal neuroepithelium (VNE)

Age (n)	VNE XS area (mm^2); mean/range	VNE length (mm); mean/range	VNE volume; mean/range
Heterocephalus glaber			
neonate (1)	0.0138	0.89	0.0139
1-week-old (1)	0.0066	0.85	0.0092
2-week-old (4)	0.0133/ 0.0049–0.0153	0.99/ 0.75–1.0	0.0136/ 0.0078–0.022
4-week-old (1)	0.0061	0.9	0.0078
adult (7)	0.0089/ 0.0056–0.0166	1.66/ 1.58–1.8	0.0148/ 0.0057–0.017
Cryptomys hottentotus			
neonate (1)	0.0364	2.05	0.062
adult (1)	0.0207	3.07	0.0533
Fukomys damarensis			
neonate (1)	0.0334	1.55	0.0436
adult (1)	0.0241	2.8	0.0641

TABLE 2. Summary of immunohistochemical labeling

Specimen ID	OMP	GAP43	Ki67
HG2 adult wild female approx. 4 year	NA	+++	NA
HG3 adult female 1 year	++	?	NA
HG4 infant P0	–	+++	+
HG5 adult male 1 year	+	+++	+
HG6 adult female 1 year	+	+++	?
HG7 adult male breeder	NA	+++	NA
HG8 adult female breeder	++	+++	?
HG9 infant P7	+	+++	+
HG10 P28 (weanling)	+	+++	+

OMP, olfactory marker protein; GAP43, growth-associated protein 43; Ki67, mitotic marker Ki67.
Reactivity: –, Nonreactive; +, sparse VSNs are reactive, by comparison to OE; ++, numerous VSNs reactive, but fewer than in OE; +++, reactivity similar to that in OE; NA, no IHC results from this specimen; ?, results unclear due to artifact (e.g., tissue folding).

DISCUSSION

Fossorial mammals possess certain highly enhanced senses to compensate for reduced visual acuity (Buffenstein, et al., 2012; Crish et al., 2006; Catania, 2005, 2019). Behavioral data indicate that chemical senses are of great importance to fossorial species, including mole rats, yet *H. glaber* is unique among rodents for the diminutive size of the adult VNE and the relative lack of VNE postnatal growth (Smith et al., 2007). In the present study, we demonstrate that postnatal growth trajectories of the VNE may be similarly static in two other African mole rats. All metric age comparisons reveal a common theme: with age, the VNE increases in length but decreases in cross-sectional area. Previously, we excluded the possibility that cell density was significantly different in subadult and adult *H. glaber*, suggesting that VNE size and neuronal numbers are truly static postnatally (Smith et al., 2007). The extent of VNE growth is likely reduced for all of these rodents and thus may be linked to their subterranean habitat. While few other subterranean mammals have been studied, in the European blind mole rat (*Spalax ehrenbergi*) there is a 7.5-fold increase in VNO length from infants to adults (Zuri et al., 1998). This provides a stark contrast to the three African mole rat species studied here, in which the postnatal increase in VNE length is less than one-fold. The three African mole rat species that we evaluated span the breadth of the Bathyergidae family tree (Faulkes et al., 2004), suggesting that the VNE traits that we observed may be those of a common ancestor.

Previously, we urged further investigation of *H. glaber* VNO to determine whether the unique lack of volume increase indicates a comparative functional deficit (Smith et al., 2007). The VNE remains recognizable across postnatal age in all of the mole rats studied. This is a contrast to some mammals in which the VNO is neuronal during prenatal development but bears no sensory neuron populations postnatally. In such species (e.g., catarrhine primates and numerous bats), the VNO is regarded as truly vestigial or even absent (Bhatnagar and Meisami, 1998; Smith et al., 2001). Components of the vasomotor pump, such as the bony/cartilaginous capsule surrounding the VNO and venous sinuses within the capsule, appear to mature at a similar postnatal schedule as in other rodents (Garrosa et al., 1998). The glandular apparatus is not fully developed at birth in African mole rats, as is the case in other rodents, and the receptor-free epithelium may be regarded as more precocious than some other rodents, as it already bears cilia in the neonate. Thus, qualitatively, there is no evidence that the VNO is vestigial in the three mole rat species studied here.

The pattern of neuron marker expression in the postnatal VNE mostly supports that position. In rats and mice, a neuronal population within the VNE is present at birth, and neurogenesis and mitosis occur within the first week, if not earlier (Nakano et al., 1990; Weiler and Benali, 2005), and our results show evidence for both markers in infant *H. glaber*. However, Ki67 reactivity in the VNE is notably lacking compared to the main MOE. Moreover, the distribution of the mitotic cells contrasts with that of the rat, in which mitotic cells cluster in the transitional zones, or poles where the VNE intersects with receptor-free epithelium (Weiler et al., 1999). A caveat is we had few samples survive the antigen retrieval process, making the findings on mitosis tentative.

Fig. 3. The VNO of newborn (**A**,**B**) and adult (**C**,**D**) mole rats at higher magnification. In the Damaraland mole rat, the vomeronasal neuropithelium (*) has numerous rows of sensory neurons in the newborn (A) and adult (C); the receptor-free epithelium (rfe) is pseudostratified columnar and likely ciliated, although the cell processes are poorly preserved in these specimens. In the naked mole rat (B,D), the vomeronasal neuropithelium appears relatively smaller in cross-sectional area compared to the Damaraland mole rat. In the naked mole rat, cilia (open arrows) are present along the simple cuboidal rfe in the newborn (A) and adult (C); microvillar processes are discernable on the apex of the neuroepithelium of the adult (small arrows, D). The rfe of the VNO is located dorsolaterally in each plate. Scale bars, 20 μm.

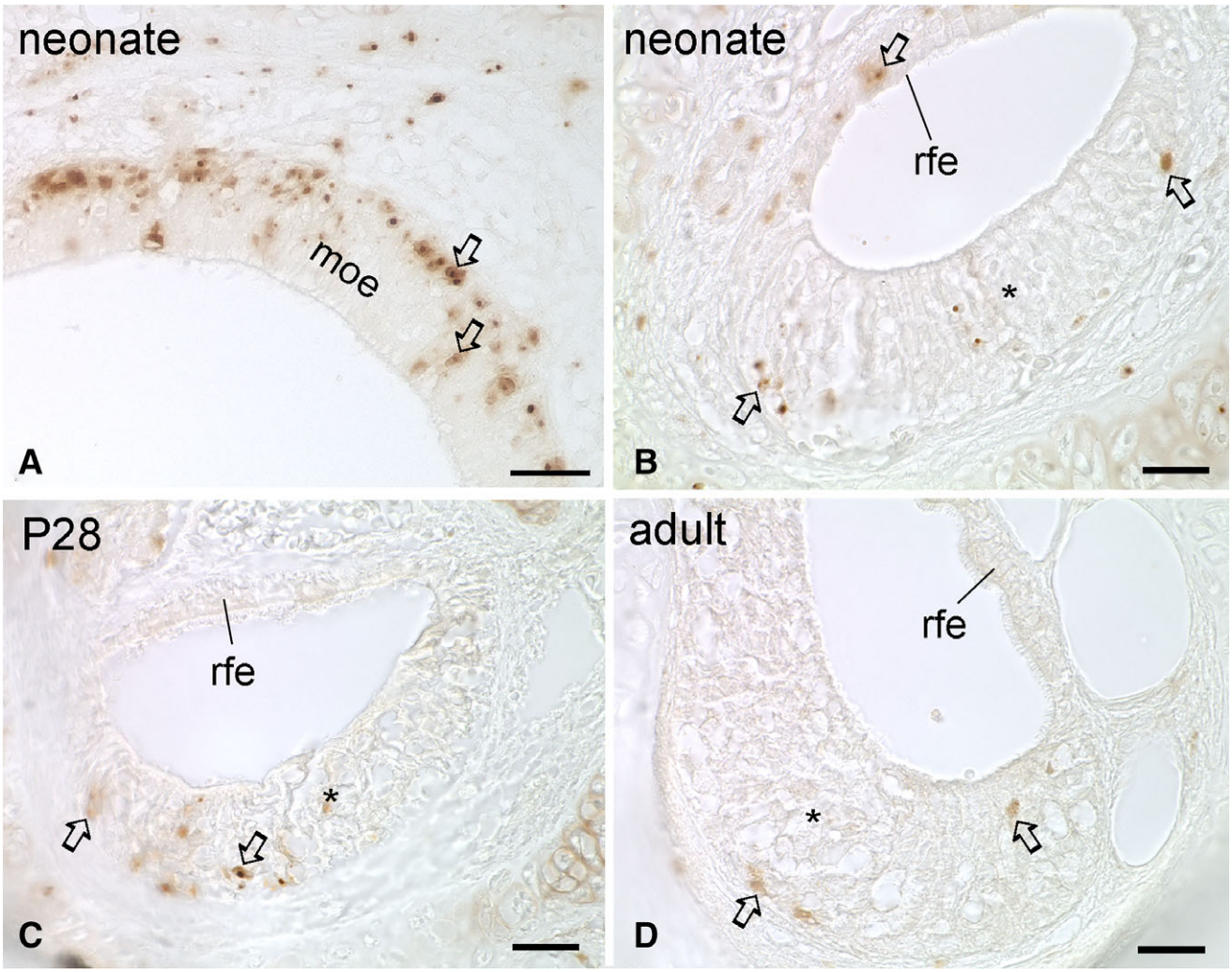

Fig. 4. Ki 67 immunoreactivity in olfactory tissues of the naked mole rat (*H. glaber*). (**A**) Olfactory neuroepithelium (moe) in newborn naked mole rat, showing numerous reactive cells (open arrows) near the base of the epithelium, and some more apically. (**B**) Vomeronasal neuroepithelium (*) of the same specimen with scattered reactive cells, more sparsely distributed compared to the moe. Note some reactive cells in the receptor-free epithelium. (**C**) A subadult P28 (weaning age) mole rat with sparse reactive cells in the vne. (**D**) An adult naked mole rat showing few reactive cells. Scale bars, (A) 50 μm; B–D, 20 μm.

A functional sensory neuroepithelium must include a neurogenic subpopulation. The *H. glaber* VNE meets this criterion. The VNE is GAP43(+) in both sexes and at all ages tested. We therefore conclude that naked mole rat VNO is neurogenic beginning at least as early as P1. Whereas our sample size is too small to make similar assertions about *Cryptomys* or *Fukomys*, the VNO is similarly organized in all of the African mole rat species and preliminary GAP43 assays indicate that the VSN is neurogenic in the neonate and the adult of both *Cryptomys* and *Fukomys* species (Smith, unpublished observations). We note that despite the apparent low Ki67 expression, GAP43 expression continues for at least 8 years, which implies that VNS precursor cells remain mitotic.

In the adult *H. glaber,* OMP is more strongly expressed in the MOE than in the VNE. The infant VNE is OMP(−). The pattern of OMP expression beginning sometime after birth and a thinner distribution in the VNE of older individuals mirrors the OMP expression pattern in rats. The rat VNE first expresses OMP on P4 and, in the adult, never expresses OMP at relative densities similar to the MOE (Farbman and Margolis, 1980).

The precise function of OMP remains unknown. Kondo et al. (2010) argued that OMP expression reflects the rate of OSN proliferation. We suggest that the mole rat VSNs may turn over before they attain terminal maturity, a case that would explain the relatively scarce OMP(+) cell population. Future work quantifying apoptosis could test that possibility. There may be a parallel to some primates of the genus *Saguinus*, which as adults possess a thin VNE that is not segregated into medial and lateral parts and is relatively OMP-deficient compared to other primates (Dennis et al., 2004; Smith et al., 2011). Further analysis to confirm that functional receptor genes are

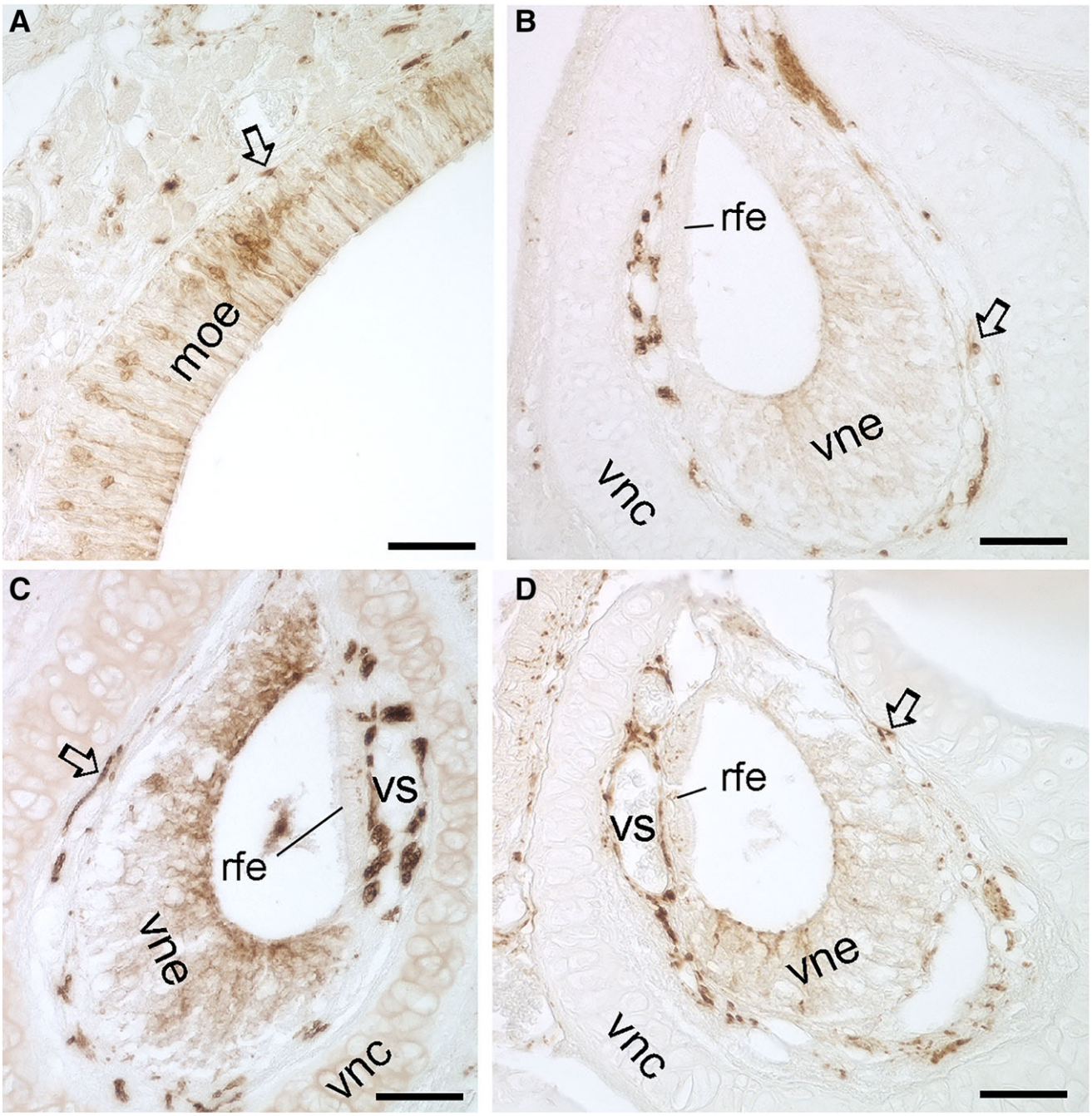

Fig. 5. Gap43 immunoreactivity in olfactory tissues of the naked mole rat (*H. glaber*). The main olfactory neuroeipithelium (moe) is shown near the dorsal extent ("roof" of the nasal fossa. (**A**) Olfactory neuroepithelium (moe) in adult naked mole rat (**B**) vomeronasal neuroepithelium (*) in newborn naked mole rat (**C**) vomeronasal neuroepithelium in P14 naked mole rat (**D**) vomeronasal neuroepithelium in adult naked mole rat. Open arrows indicate reactive axon bundles in the lamina propria. The rfe of the VNO is located dorsolaterally in each plate. VNC, vomeronasal cartilage; rfe, receptor-free epithelium. Scales, 50 μm.

present in the mole rat genome is desirable. If the receptors are intact, then subsequent work could correlate G-protein distribution with OMP expression, which would address the implicit question of whether OMP expression is necessary for function in all terminally differentiated VN sensory cells.

CONCLUSIONS

Despite the diminutive size, the VNE of *H. glaber* is both neuronal (Smith et al., 2007) and neurogenic. In this respect, its VNO is distinctive from some mammals in which VNO function is clearly lost, including numerous bats (Bhatnagar and Meisami, 1998), and Old World

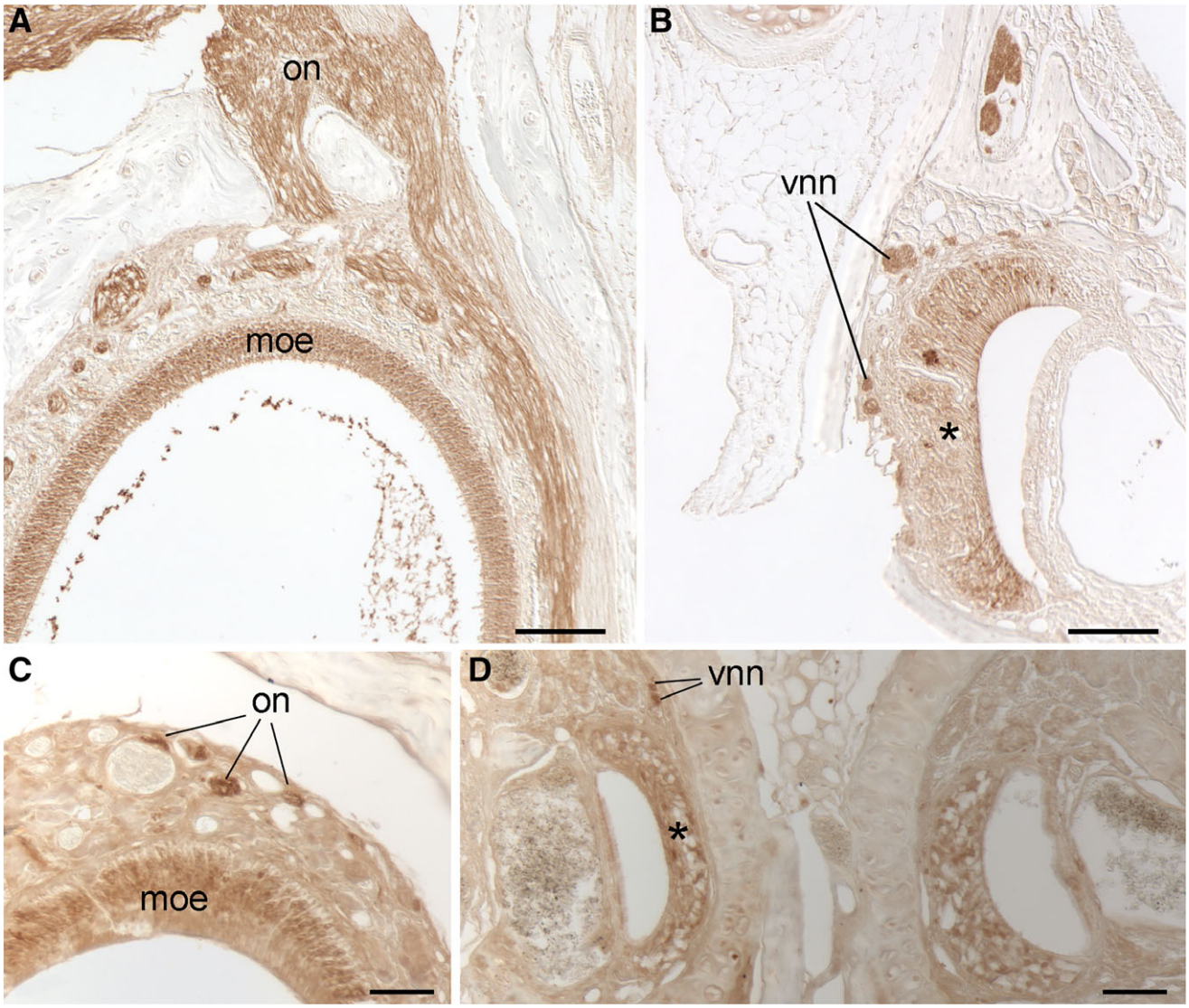

Fig. 6. OMP reactivity in an adult rat (**A**,**B**; *R. norvegicus*) and adult naked mole rat (**C**,**D**; *H. glaber*). The main olfactory neuroeipithelium (moe) is shown at the dorsal extent ("roof" of the nasal fossa. (A) In the rat, both MOE and olfactory nerves (on) and densely OMP +. (B) The rat vomeronasal neuroepithelium (*) and vomeronasal nerves (vnn), are also OMP+. Both tissues are OMP + in the adult breeding naked mole rat (C,D) although the VNE is far less reactive. The rfe of the VNO is located dorsolaterally in each plate. Scales, 100 μm.

primates (Smith et al., 2001). The human VNO vestige, which actually grows postnatally (Smith et al., 1997b), nonetheless lacks the neuronal and neurogenic nature of demonstrably functional mammalian VNOs (Bhatnagar and Smith, 2001; Witt and Hummel, 2006). Thus, the VNO of *H. glaber* and at least some other African mole rats, while unusually lacking in growth, does not appear to be vestigial. Macroscopic (e.g., osseous/cartilaginous capsule, glands, and vomeronasal nerves) and microscopic elements of a functional VNO are present.

However, the VNE may be unusually variable. Even with the relatively small sample, the overlapping volume of the VNE in subadult (0.0066–0.0153 mm^2) and adult samples (0.0056–0.0166 mm^2) is impressive. The quantitative results on the two other mole rat species indicate a similar overlap may exist. We know of no other vertebrate that exhibits the same lack of ontogenetic size disparity in VNE volume. Moreover, the coefficient of variation in the adult VNE volume in *H. glaber* seems extremely high, at 43%. This may partly be due to the small sample size. However, this coefficient for VNE volume in similar sized samples of voles ranges from 14% to 25% (Maico et al., 2003).

Unfortunately, an experimental understanding of the vomeronasal system in mole rats is completely lacking. Behavioral evidence is our sole point of reference, and in this respect, it may well be significant that certain major functions attributed to the vomeronasal system of other

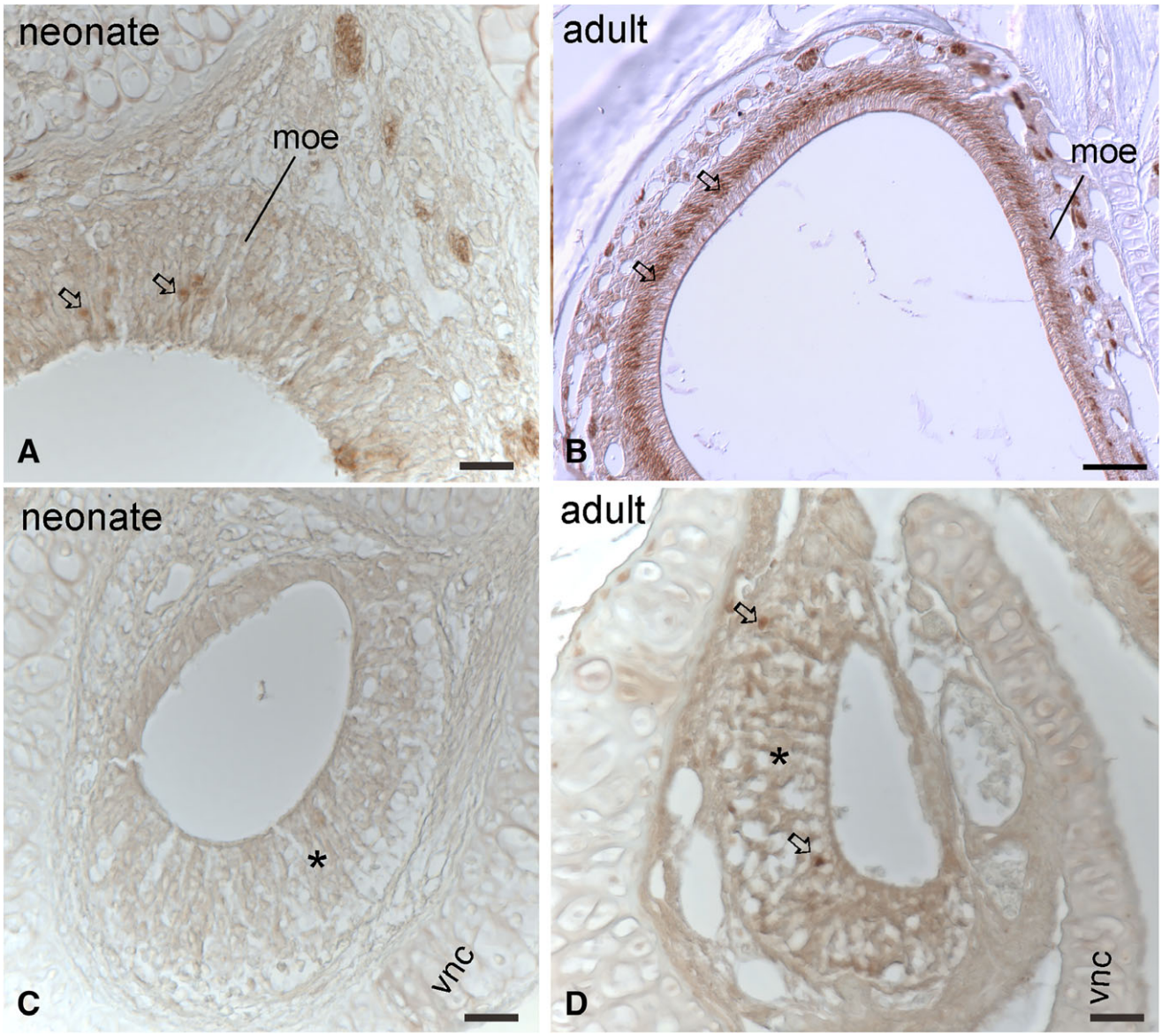

Fig. 7. OMP reactivity in a P1 (**A**,**C**) and adult (**B**,**D**) naked mole rats. The main olfactory neuroeipithelium (moe) is shown at the dorsal extent ("roof" of the nasal fossa. A) at birth the main olfactory neuroepithelium (moe) contains sparse reactive olfactory sensory neurons (open arrows) while in adults (B) the moe is highly reactive, similar to the rat. (C) At birth the vomeronasal neuroepithelium (*) is OMP -, while in adults (D) the neuroepithelium of most naked mole rats contains spare reactive sensory neurons (open arrows). The rfe of the VNO is located dorsolaterally in each plate. Scales, A, C, D, 50 μm; B, 100 μm.

rodents, such as reproductive suppression, are instead behaviorally reinforced in *H. glaber*. At this point, we can only speculate that a reduced functional role may translate into reduced selection pressure on the vomeronasal system. If so, African mole rats may provide a useful genetic model for understanding the great morphological variability in the mammalian vomeronasal organ (e.g., Weiler et al., 1999; Smith et al., 2011, 2014).

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