RESEARCH ARTICLE

Species Specific Structural Differences of the Main Olfactory Bulbs in Dog (*Canis Familiaris*) and Goat (*Capra Hircus*)

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Abstract

The structural characteristic of the main olfactory bulb (MOB) shows species-dependent variations. However, the correlation of such structure with olfactory function as well as with the ecological and evolutionary behaviours is less understood. Therefore, the aim of the current investigation was to elucidate the species differences of the structure of MOB in two ecologically diverse species, dogs and goats. For that purpose, thirteen heads of each species were obtained. The brains and the olfactory bulbs were dissected for anatomical, histological and immunohistochemical analyses for detection of glial fibril acidic protein (GFAP) and chromogranin A. Anatomically, the volume of the olfactory bulb to the whole brain volume was higher in dogs (1.74%) than goats (0.57%). Histologically, the main olfactory bulbs were organized in the following layers: the olfactory nerve layer (ONL), the glomerular layer (GL), the external plexiform layer (EPL), the mitral cell layer (MCL), internal plexiform layer (IPL) , the granule cell layer (GCL) and a periventricular layer (PVL). Enormous number of juxtaglomerular and mitral cells, thickness of GL, EPL and GCL were significantly greater in dogs than goats. Immunohistochemically, the integrated density percentage of GFAP and chromogranin A was significantly higher in dogs than goats. The average number of mitral cell neurons in a standard area was significantly higher in dogs (6 ±.37) than goats (3.16 ±.31). Therefore, our data suggested that such apparent structural difference of MOB between the dog and the goat could be correlated with superior olfactory sensitivity and subsequently the ecological and lifestyle behaviour of dogs.

Keywords: Main Olfactory bulb, Immunohistochemistry, Mitral cell, Olfactory glomeruli.

Introduction

The olfactory sense is unique, special and playing a major role in general animal awareness. It is used for find, recognize and discriminate between foods, communicate, interact, navigate, recognize, predator avoidance, mating, and territoriality [1,2].

Receiving and processing of odor occur by the olfactory system either main or accessory one. The main olfactory system comprises the main olfactory mucosa and bulb [3]. Chemoreceptors that located in the mucosa of nasal and oral cavities can differentiate the flavor of different foods [2]. The main olfactory bulb (MOB) is the first complex (multilayered) structures which receive odor stimuli and relay it directly to the central nervous system by the olfactory receptor cells (ORNs) in the olfactory epithelium. These stimuli proceeds in a hierarchical manner into the olfactory bulb through the synapse of ORNs (primary neurons) axons with mitral and tufted cells (secondary neurons) dendrites within the olfactory bulb glomeruli [4-9]. The MOB is composed of various types of main neurons as mitral and tufted (M/T) cells and interneurons as the periglomerular cells, granule cells and glial cells. The latter cells are detected by using antibodies against GFAP

(glial fibril acidic protein as intermediate filament protein) in their cell processes [10]. The synapses occur between different neurons of the olfactory bulb are identified by using antibodies against chromogranin A [11].

The dogs possess so amazing sense of smell (primary special sense) using it for a wide range of activities as catching the prey, in the detection of narcotics and contraband agriculture products, detect drugs, search for lost individuals, homicide victims, and forensic cadaver materials [2,12]. Also, the goats have a low dependency on their sense of smell for only grazing and reproduction [13,14]. So, olfaction is varied between animal species according to their habits, one of them is the feeding habit as the dog is a carnivore but the goat is a herbivore. Experimental work on the dogs has exposed the presence of a high olfactory acuity, which has emerged as essential to their evolutionary success [12]. However, there is a shortage of structural data to support the enhanced sensitivity of olfactory systems in dogs. Most studies have focused on the olfactory bulb structure of the rodents [15,16]. Few studies has explored the olfactory bulb structure in dog and goat and corelate the result to the feeding behaviour of the animals [17]. Therefore, the purpose of the current investigation was to compare the structure characteristic of the olfactory bulb among two animal species; one with higher olfactory capability as dog and the other with lower as goat using anatomical, histological and immunohistochemical studies (including the localization of GFAP and chromogranin A expression). Moreover, the possible correlation between the structure of the MOB with the ecological behaviour of each animal species was investigated.

Materials and Methods

Animals and ethical considerations

This study was conducted on 13 adult Balady female dogs (*Canis familiaris*) and the same number of adult female goats (*Capra hircus*). The dogs were purchased from the laboratory animals research Center, Faculty of Veterinary Medicine, Zagazig University, Egypt. The animals were then kept under observation for 2 weeks before the beginning of tissues harvesting. The goats' heads were taken from local abattoirs in Zagazig, Egypt. The protocol in this research has been reviewed and approved by the Institutional

Animal Care and Use Committee (IACUC), Zagazig University (ZUIACUC/ 2/F/57/2018).

Specimens harvesting

The dogs were sedated by intramuscular (IM) injection of 0.05-0.15 ml / kg body weight (BW) of xyla-ject (Adwia Pharmaceuticals Co, Egypt). The animals were then anesthetized by intravenous (IV) injection of 20-25 mg/Kg BW of thiopental (Pentothal; Hospira Healthcare Corporation) [18]. After that, the animals' common carotid arteries were cannulated then perfusion was done with 4% paraformaldehyde (PFA) in 0.1M phosphate buffer for light microscopy and immunohistochemistry. At the time of dissection, the head was cut off and the lower jaws were removed. The skulls of the animals were removed using a bone saw to expose carefully the brains and the bulbs especially in goats. Finally, the brains of both dogs and goats were dissected from the cranial cavities.

Scaling the volume of the olfactory bulb with the whole brain

To examine the scaling of the OB with the brain, we utilized 6 dogs' and 6 goats' formalin-fixed brains with the olfactory bulbs. The volumes of the whole brain (VWB) and the olfactory bulb (VOB) were evaluated by the volumetry of water immersion protocol as mentioned previously [19]. In which the brains of both dogs and goats were rinsed in physiological saline and then suspended from a stick connected to a stand with a good thread. The brain was then submerged in an electronic analysis of a 50 mL beaker containing physiological saline. Care was done to guarantee that the beaker's internal surface was not touched by the brain. When the brain sample was soaked, the change in weight (in grams) of the physiological saline corresponded to its volume (in milliters) assuming the specific gravity of the physiological saline is equal to one. The OBs were at that time dissected from the brains and their volumes were calculated by the same method. Finally, comparisons of VOB with whole brains VWB were expressed as ratios (%). These ratios (%) were calculated using the following formula: VOB/ VWB×100. The correlation coefficient between the volume of olfactory bulbs (VOB)

and whole brains (VWB) were statistically measured in both dog and goat.

Tissue preparation for histological and immunohistochemical analyses

Dissected OBs of paraformaldehyde perfused fixed brains from 6 dogs and 6 goats were immediately immersed with 4% paraformaldehyde in 0.1 mole phosphate buffer overnight at 4°C then processed till obtaining paraffin sections. Some paraffin sections were deparaffinized and hydrated, followed by staining with haematoxylin and eosin (H&E) for histological analysis [20]. The other deparaffinized sections were utilized for immunohistochemical analysis as described elsewhere [21,22]. Such sections were hydrated then washed in phosphate buffer saline (PBS) at pH 7.2 for 5 min. To block the endogenous peroxidase activity, these sections were immersed in absolute methanol containing 0.3% H_2O_2 followed by rinsing with water and incubation with 10% normal goat serum (blocking reagent) at room temperature for 1h to reduce the non-specific binding of immunoglobulins. Then the sections were incubated overnight with the primary antibody dilutions: rabbit polyclonal anti-GFAP (Glial Fibrillary Acidic Protein) (1:1000, Cat. No.ab7260, Abcam,Cambridge, United States) and rabbit polyclonal anti-Chromogranin A (1:200, Cat. No. ab45179, Abcam, Cambridge,United States).

The specificity of primary antibodies for dog and goat tissues was validated by using normal rabbit IgG on goat and dog nervous tissues by concentration parallel to the concentration of the primary antibodies as a negative control. Then, the primary antibody was incubated at 4°C overnight. After washing with PBS, the sections were incubated with biotin-conjugated goat anti-rabbit IgG antiserum (Histofine kit, Nichirei Corporation, Tokyo, Japan) for 60 min. Then washed in PBS, followed by incubation with streptavidin-peroxidase conjugate (Histofine kit, Nichirei Corporation, Tokyo, Japan) for 30 min.

The streptavidin-biotin complex was visualized with 3, 3′-diamino-benzidine tetrahydrochloride (DAB)-H_2O_2 solution, pH 7.0, for 3 min. The sections were then washed in distilled water and Mayer's hematoxylin was used as a counterstain. Micrographs of the sections were taken with a digital camera (Leica EC3, Leica, Germany) connected to a microscope (Leica DM500, Germany).

Morphometric analysis

For the morphometric study, six animals were utilized per species. For measuring the thickness of olfactory bulb layers, we used the Image J analysis software (Fiji image j; 1.51 n, NIH, USA) and representative fields scattered in the captured photomicrographs at 40× magnification that were selected depends on the presence of well-defined olfactory bulb layers at cross-sections of the 6 animals/species. The number of mitral cells was also counted per standard field area (49472μm) at 400× magnification. In addition, the integrated density percentage of GFAP and chromogranin A immuno-positive expressions were also measured in the captured representative immunohistochemical fields at 40× magnification as mentioned previously [23]. In brief, we used immunohistochemical images wherein the brown areas were positive reactions stained by DAB. The color deconvolution was then applied to these images via H DAB matrices of an image J software. On DAB matrices, the images were changed to grayscale image (Type − 8bit) then the threshold was adjusted to detect only the DAB positive according to the intensity. The threshold parameter was constantly throughout analysis for all immunohistochemical images. The average integrated density percentages for both species were reported.

Statistical analysis

To analyze the numerical values obtained from both dogs and goats, we used the Student's t-test in the Statistical Package for the Social Sciences (SPSS) software program (version 16.0; Chicago, USA). Data were expressed as means ± standard error (SE) , correlation coefficient and statistical significance were determined at 95% confidence interval (p ≤ 0.05), n= 6/ group.

Results

Anatomical features and volumes of the olfactory bulbs

The olfactory bulbs (OBs) passed as two separated segments in the anterior aspect of the cerebral hemispheres and connected to them via olfactory tracts. They were clearly seen in the ventrolateral and ventral views in dogs and goats, respectively (Figure 1A, B). The dissected MOB of the dog was semi-circular in shape with a ventrolateral rough surface. Meanwhile, the goat's bulb was elongated in shape with only a rostral ventral rough surface (Figure 1C, D).

Sagittal planes through MOBs appeared multilayered concentrically arranged as onion-like and their layers on both dorsal and ventral sides with hardly detected olfactory ventricle in the dog. However, only one ventral side of non-uniformly thickness with a clearly demarcated olfactory ventricle was detected in goat (Figure 1E, F). The VOB was statistically higher in the dog (1.3 ± 0.07 mm^3) than in the goat (0.65 ± 0.04 mm^3). The ratio of the VOB with the VWB was statistically greater in dogs (1.74 ± 0.13) than in goat (0.57 ± 0.041). The VOB in the dog was larger than the goat when correlated with the whole brains (strong negative linear relationship between them), r = -0.9, $P \leq 0.05$, (Table 1).
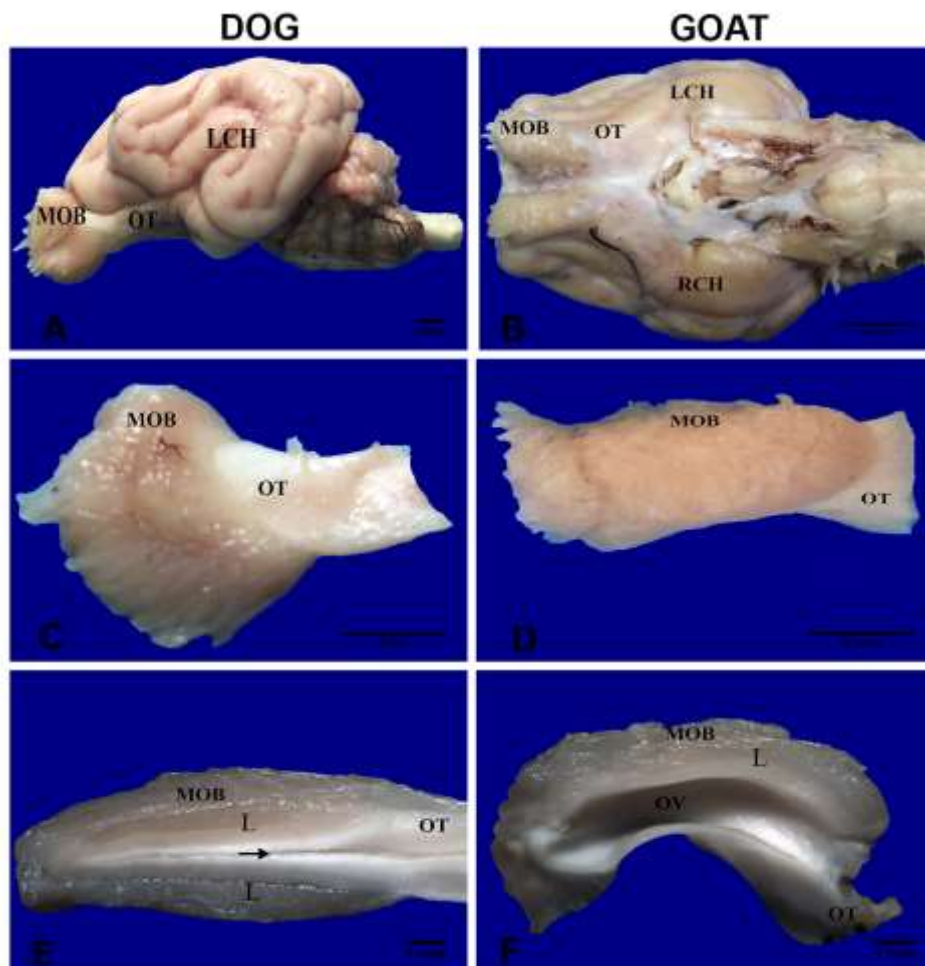
Figure 1: Anatomical features of the main olfactory bulbs from both dogs and goats. The main olfactory bulbs (MOB) lying in ventrolateral (A) and ventral positions (B) in dogs and goats, respectively. The shape of MOB is semi-circular in the dogs with a rough ventrolateral surface (C) and elongated in goats with a rough ventral surface (D). Sagittal planes in MOB display their segments (L) on both dorsal and ventral sides in dogs and only on ventral side in goats, with hardly detected olfactory ventricle "arrow" in the dogs (E) and clearly demarcated one (OV) in the goats (F). Olfactory tract (OT), Left cerebral hemispheres (LCH) and Right cerebral hemispheres (RCH) were observed.

Table 1: Scaling the volumes of the olfactory bulbs with whole brains in both dogs and goats. Values are means (mm^3) ± SE^1, (N^2=6).

Animals / Criteria	Dog	Goat	Sig (2-tailed)
VOB^3	1.3±0.07[4]	0.65±0.04	0.00
VWB^5	74.3±1.9	114.7±1.1	
Ratio: VOB to VWB (%)	1.74±0.13	0.57±0.041	

[1] Standard error; [2] the number of animals; [3] the volume of the olfactory bulbs; [4] indicate statistically significant differences (P ≤ 0.05) and [5] the volume of the whole brains.

Histological features of the MOBs

Histologically, H&E stained MOBs sections were shown in Figure 2 for determining MOBs' layers in both animal species. From outsides toward insides, the MOBs were organized in the following layers: the olfactory nerve layer (ONL), the glomerular layer (GL), the external plexiform layer (EPL), the mitral cell layer (MCL), internal plexiform layer (IPL) , the granule cell layer (GCL) and a periventricular layer (PVL). IPL is well clear in the dog but not distinct in the goat. Moreover, the ependymal layer (EL) that lining the olfactory ventricle was founded only in the goat.
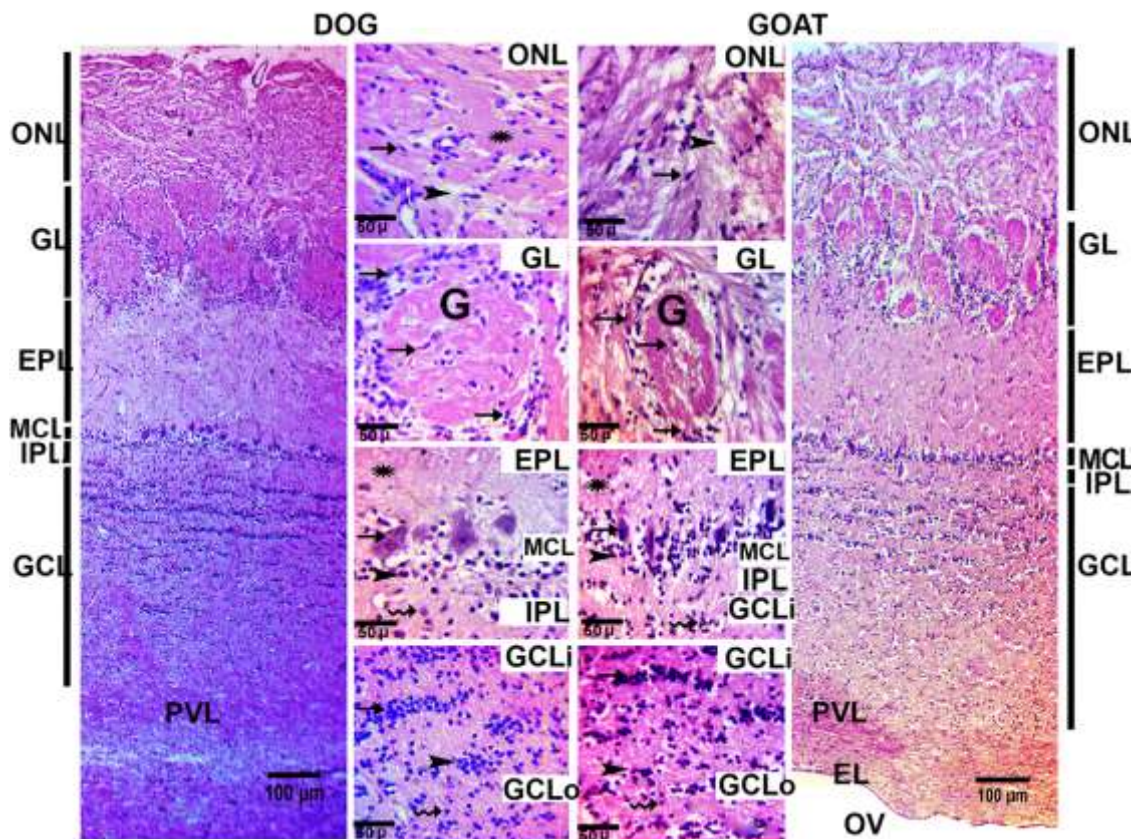

Figure 2: Histological features of the main olfactory bulb layers. Hematoxylin and eosin (H&E) stained sections from both dogs and goats showing the olfactory nerve layer (ONL), glomerular layer (GL), external plexiform layer (EPL), mitral cell layer (MCL), internal plexiform layer (IPL), granule cell layer (GCL), the periventricular layer (PVL), the ependymal layer (EL) and olfactory ventricle (OV). Higher magnifications from both dogs and goats showing ONL of primarily olfactory nerve bundles (asterisks) that ensheathed by glial cells of ovoid cell bodies (arrows) and pale cytoplasm (arrowheads), GL with glomeruli (G) and juxtaglomerular cells (arrows), EPL neuropil (asterisks), MCL with single row of mitral cell bodies (arrows) surround with numerous granule cell bodies (arrow heads), IPL neuropil with dispersed spherical cell bodies (zigzag arrow) and GCL neuropil with cell bodies in form of fusiform bands (arrows) in the inner part (GCLi) then arrange in clumps (arrowheads) of several cells followed by a few cells and finally individual (zigzag arrows) in the outer parts (GCLo).

The ONL constituted the outermost layer and formed primarily of olfactory nerve axons that ensheathed by olfactory ensheathing glial cells (OECs) of ovoid cell bodies and pale cytoplasm. Numerous processes of OECs encircle a large number of olfactory nerve axons in the dog. However, few processes of OECs were enclosed a few numbers of olfactory axons in goat. These olfactory axons gathered together to constitute olfactory nerve fascicle.

The GL lied just underneath ONL and enclosed spherical masses- like structures called glomeruli. They crossed the entire perimeter of the olfactory bulb. In the dog, the GL had large size glomeruli (450–460 µm diameter) that occurred mostly single or in pairs. In the goat, the glomeruli were small in size (290–400 µm in diameter) and usually arranged in a double layer. A large number of juxtaglomerular cells were in close association and entered the glomerular neuropil. These cells were surrounded by the entire glomeruli in the dog, but they were mainly detected on the lateral and ventral aspects of the goat's glomeruli.

The EPL of both species was the second biggest layer situated between the GL and MCL. The EPL was manifested by diffusely distributed tufted cells throughout the whole layer. The neuropil of EPL formed from the dendrites of mitral cells, tufted cells, and granule cells.

The MCL considered the thinnest layer that contained a single row of mitral nerve cells and numerous interneurons of spherical cell bodies. Mitral nerve cells were large and pyramidal in shape with dark basophilic stained cytoplasm which housed single central spherical nuclei in the dog. However, mitral cells were small and elongated with central ovoid nuclei in the goat. The difference in the number of mitral cell neurons between both animals was significant (P ≤ .05) as the average number of mitral cell neurons in a standard area was 6 ±.37 and 3.16 ±.31 in dog and goat, respectively.

The IPL was deep to the MCL and considerably thinner compared to the EPL. In the dog, this layer had neuropil (axons of the mitral cells and dendrites of the granule cells) and granule cell bodies that arranged individually, in pairs or in the linear cluster. On the contrast, the IPL layer was hardly demarcated in the goat.

The GCL was the deepest and biggest layer of the MOB in both species. This layer characterized by a large number of round to ovoid granule cells. According to the density and arrangement of the granule cells, the GCL could be divided into inner and outer sub-layers.

Regarding the inner sub-layers, the cells were arranged in clusters of several cells, then of a few or individual cells. The cells of the outer sub-layers set very near to each other constituting long, overlapping, fusiform bands that are longer in the dog than the goat. The goat had larger spaces in between cell bands than in the dog.

The PVL (sub-ependymal) layer present surrounding the entire circuit of the olfactory ventricle in the goat and fills the entire center of the bulb in the dog. The layer had a few numbers of randomly arranged cell bodies and a large number of tangentially directed axons.

The EL was detected only in the goat as a last thin layer of the MOB and bordered the lumen of the olfactory ventricle. This layer possessed a very high density of nerve fibers and few cell bodies. There are variations in the thickness of olfactory bulb layers especially the GL, EPL, and GCL, which were significantly greater in the dog than the goat P≤ 0.05, (Table 2).

Table 2: Comparison the thickness of the main olfactory bulb' layers of dog and goat. Values are means (µm) ± SE[1], (N[2]=6).

| Animals MOBs' [3] layers	Dog	Goat	Sig (2-tailed)
Olfactory nerve layer	602.07±302	504.95±166.97	0.47
Glomerular layer	315.5±31.7 [4]	244.9±39.8	0.04
External plexiform layer	346.95±37.5 [4]	304.2±52.5	0.02
Mitral cell layer	33.3±2.6	31.64±1.64	0.17
Internal plexiform layer	53.6±8.3	-	-
Granule cell layer	568.1±12 [4]	732.83±74.11	0.000

[1] Standard error; [2] the number of animals; [3], Main Olfactory Bulb; 4 indicate statistically significant differences (P ≤ 0.05).

Immunohistochemical analysis

We studied the expressions of the GFAP and chromogranin A in the layers of MOBs from both animal species (Figure 3).

Concerning GFAP-immunostained sections, localizations of positive GFAP-immunoreaction in the cytoplasmic processes of neuroglia cells were heterogeneous in the MOBs' layers in both dog and goat. GFAP-immunoreaction was clearly expressed in the inner portion of the ONL, periglomerular regions of the GL and was poorly expressed in the glomerular neuropil, EPL, PVL, and the EL. Statistically, the mean value of integrated density percentage of the GFAP immuno-positive reaction in dog was 37.79 ± 0.29 recording a significant increase when compared with the goat (31.08 ± 0.34).

To demonstrate the main neurons of the MOBs (mitral and tufted cells), the immunohistochemical staining with anti-chromogranin A was performed for MOBs in both animal species. A positive chromogranin A- immunoreaction was localized to somata of tufted and mitral cells. Such tufted cells were distributed in the periglomerular region, middle cells in the center of the EPL and above the mitral cell. Also, mitral cells' dendrites forming neuropil of the EPL and mitral cells' axons forming neuropil of the IPL displayed immunoreactivity in both dog and goat. Dog's tufted cells were pronounced than goat. The mean value of integrated density percentage of positive chromogranin A immunoreaction throughout MOBs' layers was significantly amplified in the dog (28.10 ± 0.44) than goat (22.79 ± 0.31).
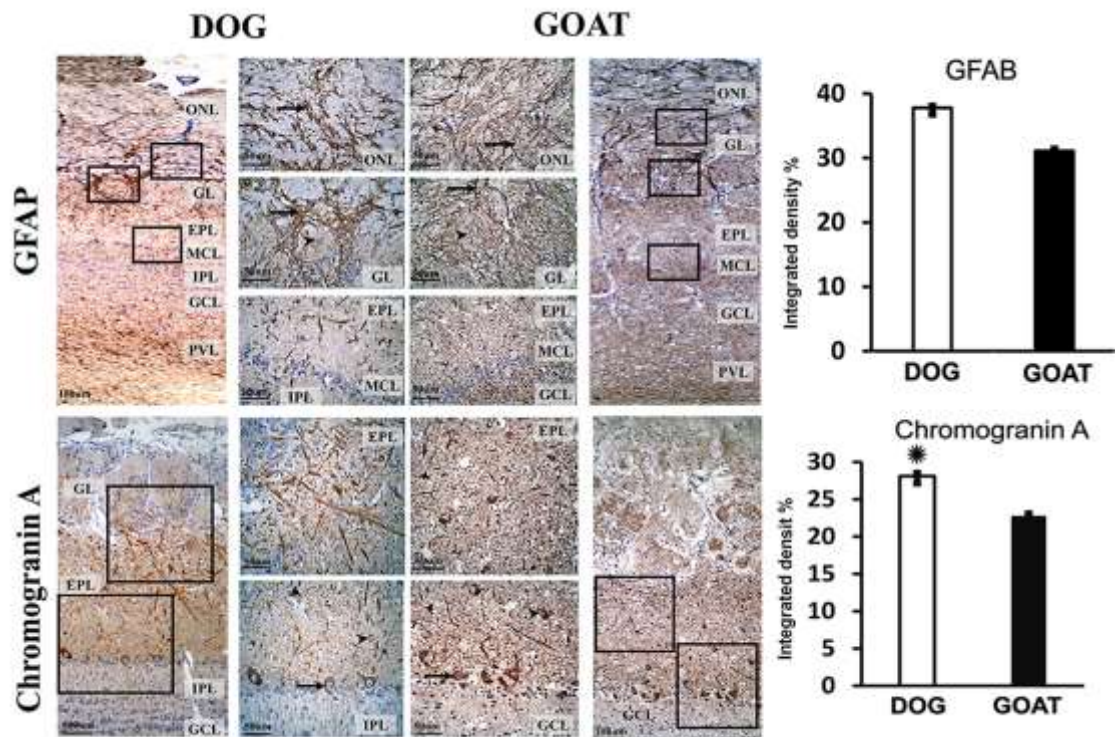
Figure 3: Immunohistochemistry staining for glial fibril acidic protein and chromogranin A in the main olfactory bulbs from both dog and goat. Positive GFAP immunoreactions are clearly expressed in the inner portion of the ONL (arrows), periglomerular regions of the GL (arrows) and poorly expressed in the glomerular neuropil (arrowheads), EPL, PVL, and the EL. Positive chromogranin A‑ immunoreactions are localized to somata of the mitral cells (arrows), tufted cells (arrowheads) and mitral cells' dendrites (part of neuropil in the EPL) and mitral cells' axons (part of neuropil in the IPL) in both dogs and goats. Brown color indicates a positive reaction. Bar charts demonstrating integrated density percentages of the GFAP and chromogranin A positive expressions in all the OB layers of both dogs and goats. Data are expressed as means ± SE (n=6), * indicate statistically significant differences at (P ≤ 0 .05) as determined using independent student's t-test. GFAP, glial fibrillary acidic protein; OB, olfactory bulb; ONL, olfactory nerve layer; GL, glomerular layer; EPL, External plexiform layer.

Discussion

We hypothesize that the variations of MOBs' cytoarchitecture correlate with the olfactory functional challenges in both studied species and consequently their lifestyles. Therefore, our study was designed to focus on the differences in MOBs of bothdogs (carnivore) and goats (herbivore), based on anatomical, histological, immunohistochemical and morphometrical analyses in relation to differences in olfactory functional needs.

In current study, MOBs' positions were differed in both species. MOBs were obviously seen at ventrolateral and ventral brain views in dog and goat, respectively.

These findings are similar to those described in a previously published study [24] that the adult African grass cutter olfactory bulbs were visible on the dorsal brain view but the elephant olfactory bulbs were hidden under the frontal lobes [25].

Large number of olfactory nerve entered the MOBs at the ventrolateral surface in dog. Meanwhile, only at rostral part of goat giving this surface the rough appearance. These are indicative for large number of olfactory nerve that enters the MOBs. Furthermore, the volume of the olfactory bulbs was greater in dogs than goats and MOB segments present on both dorsal and ventral sides in the dog and only on the ventral side in goat. This difference in volume and segment distributions

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may be suggestive for the different olfactory abilities of both species. The size of the olfactory bulb decreases in relation to the decrease in the functional needs of the olfaction [17,26,27]. These reflect the better olfactory function of the dog than the goat. We also found that MOB of dog lacks olfactory ventricle, but was large and clear in goat [28]. There are no described functional olfactory differences between animals in which the ventricle is either present or absent [28].

In the investigated dog, as in mammals, the basic histological organization of the MOB was with 7 layer; ONL, GL, EPL, MCL, IPL, GCL and PVL is constant. Some studies recorded the same result in the African elephant [29], but other studies described 6 layers in rats [16]. In addition, IPL of the examined goat was ill-distinct layer and this is compatible with a previous study in sheep [30] and other numerous studies in the elephant [29,31].

The goat had PVL and EL unlike the dog had only PVL that possessed the progenitor of many olfactory bulb cells [32].

The GL is considered the functional units of the MOB as the synaptic contact between olfactory receptor axons and M/T cells occur in it [33]. Therefore, species differences were observed in the GL, particularly the olfactory glomeruli structure is complex in animal with well-developed olfactory abilities, as described previously in elephant [29]. In contrast, species whose olfactory senses are mediocre developed, such as the domestic chicken (*Gallus gallus*), showed ordinary glomerular structure [34,35].

Our results clarified that the GL consisted of olfactory glomeruli and periglomerular regions. The olfactory glomeruli in goat were small in size and arranged in double layer unlike the dog, were larger and arranged in one layer. This feature is characteristic in many mammals, but it may reach up to 4 layers as in the African elephant [29]. It is interesting that, the large number of juxtaglomerular and glia cells was detected around and within the olfactory glomeruli of dog; this may have interesting implications for olfactory information processing. On contrast,

these cells were lesser in goat as reported in birds and fish [36], which did not have a well-developed sense of smell.

Odor signals were processed within the olfactory glomeruli then transmitted in the EPL along the primary dendrite of M / T cells [4]. The EPL of both examined species is mostly occupied by diffusely distributed tufted cells and dendrites of M/T and granule cells. This layer is statistically greater in thickness in the dog than the goat. These may be contributed for more odor propagation occur along the primary dendrite of M/T cells [38,39].

Mitral cells are one of the MOB' principal neurons and their large somata were constituting the MCL of both studied species. The somata of mitral cell were pyramidal in shape with central spherical nucleus in the dog and small elongated with a central ovoid nucleus in the goat. These result proved that mitral cell somata has different shapes according to the animal species [8,26]. There was a significant difference in the number of the mitral cell between both species. As a result of the importance of mitral cells in olfactory signal transduction to higher brain centers, this difference may be the reason for the high odor sensitivity in dogs than goat [26].

The GCL is the largest layer of the MOB [5]. GCL was very large in the investigated dog when compared to the goat and contained aggregates of tightly packed granule cell somata. They synchronize their function via gap junctions between them [32].

Our most important results of the ONL are those of the olfactory nerve axons and OECs, particularly OECs processes, which are not uniformly distributed in the ONL of both species. Specifically, positive GFAP-immunoreaction is mostly restricted to the inner portion of the ONL. Within this portion, the OEC followed the olfactory axons that run in complex courses and loosely arranged. These results confirmed those obtained previously in mouse [10] and rat [16] suggesting that the glial process distribution divide the ONL in to outer and inner sub-lamina in both species and this may help be to

potentiate signaling from the olfactory receptor cells.

In this study, GFAP positive reactions were strongly expressed in GCL beside PVL in the dog more than the goat, which is typical of results obtained by another study [16], which suggested that glial cells processesed help in the lateral migration of newly generated cells from the PVL (the rostral migratory stream) to the olfactory bulb, or facilitate radial migration of new cells to the GL where synaptic transmissions occur.

Mitral and tufted cells share certain immunohistochemical properties. For instance, their immunopositive reactivity with chromogranin A. The latter is a prominent neuropeptide of the olfactory bulb and biosynthesized in the somata of the M/T neurons and periglomerular region of the olfactory bulb [11]. It acts as a calcium-binding protein, participating in calcium sequestration and regulation of osmotic pressure within chromaffin vesicles [40]. Consequently, tufted cells are considered as smaller mitral cells and categorized into a single group termed M/T cells as previously mentioned [8].

Our result showed that chromogranin A were differentially dispersed in the MOB neurons and might perform specific functional roles. Moreover, the integrated density percentage of their immunoreactivity was significantly augmented in dog than goat to indicate higher olfactory cue in dogs.

Conclusion

In dogs, higher olfactory bulb volume to whole brain volume was observed. The presence of both MOB's segments, large olfactory glomeruli with prominent variable synapses, large numbers of juxtaglomerular and mitral cells, and the prominent IPL has been associated with excellent olfactory abilities of smell, when compared to goats with less olfactory abilities. These structural variations in both species reflect on the olfactory functional challenges and subsequently the ecological and lifestyle behaviors of these species. Dogs have the ability for processing olfaction, distinguishing various odors, and long-time memory of odors

is much stronger than that of goats. Moreover, our study provides background information for future work comprising more species and advanced techniques.

Conflict of interest

There is no conflict of interest.

References

[1] Prasad, B.C. and Reed, R.R. (1999): Chemosensation: molecular mechanisms in worms and mammals. Trends Genet, 15(4): 150-153.

[2] Padodara, R. and Jacob, N. (2014): Olfactory sense in different animals. Indian J Vet Sci, 2(1):1-14.

[3] Meyerhof, W. and Korsching, S.(2009): Chemosensory systems in mammals, fishes, and insects. Preface. Results Probl Cell Differ, 47: v-xi.

[4] Greer, C.; Whitman, M.; Rela, L.; Imamura, F. and Gil, D.R. (2008): Architecture of the olfactory bulb in olfactory and taste 4th volume of the Senses: A Comprehensive Reference,1st edition. Elsevier . 623-640.

[5] Kosaka, T. and Kosaka, K.(2009): Olfactory Bulb Anatomy, in: Squire, L.R, 7th edition . Encyclopedia of Neuroscience. Academic Press, Oxford. 59-69p.

[6] Kratskin, I.L. and Belluzzi, O. (2003): Anatomy and neurochemistry of the olfactory bulb. In: Doty RL (ed) Handbook of olfaction and gustation, 2nd edition. Marcel Dekker, New York, 235–276p.

[7] Field, P.; Li, Y. and Raisman, G.(2003): Ensheathment of the olfactory nerves in the adult rat. J Neurocytol, 32(3): 317-324.

[8] Nagayama, S.; Homma, R. and Imamura, F. (2014): Neuronal organization of olfactory bulb circuits. Front Neural Circuits, 8: 98

[9] Louca, A.L. (2018): The Olfactory Bulb, In: Shackelford, T.K., Weekes-Shackelford, V.A. (Eds.), Encyclopedia of Evolutionary Psychological Science. Springer International Publishing, Cham, 1-5p.

[10] Au, W.W.; Treloar, H.B. and Greer, C.A. (2002): Sublaminar organization of the mouse olfactory bulb nerve layer. J Comp Neurol, 446(1): 68-80.

[11] Bergmann, M.; Schuster, T.; Grabs, D.; Marqueze-Pouey, B.; Betz, H.; Traurig, H.; Mayerhofer, A. and Gratzl, M. (1993): Synaptophysin and synaptoporin expression in the developing rat olfactory system. Brain Res Dev Brain Res, 74(2): 235-244.

[12] Craven, B.A.; Paterson, E.G. and Settles, G.S. (2010): The fluid dynamics of canine olfaction: unique nasal airflow patterns as an explanation of macrosmia. J R Soc, Interface, 7(47): 933-943.

[13] Gelez, H. and Fabre-Nys, C. (2004): The "male effect" in sheep and goats: a review of the respective roles of the two olfactory systems. Horm Behav, 46(3): 257-271.

[14] Sanon, H.O.; Kaboré-Zoungrana, C. and Ledin, I. (2007): Behaviour of goats, sheep and cattle and their selection of browse species on natural pasture in a Sahelian area. Small Ruminant Res, 67(1): 64-74.

[15] Onyono, P.N.; Kavoi, B.M.; Kiama, S.G. and Makanya, A.N. (2017) : Functional Morphology of the Olfactory Mucosa and Olfactory Bulb in Fossorial Rodents: The East African Root Rat (*Tachyoryctes splendens*) and the Naked Mole Rat (*Heterocephalus glaber*). Tissue Cell, 49(5): 612-621.

[16] Olude, M.A.; Ogunbunmi, T.K.; Olopade, J.O. and Ihunwo, A.O. (2014): The olfactory bulb structure of African giant rat (*Cricetomys gambianus*, Waterhouse 1840) I: cytoarchitecture. Anat Sci Int, 89(4): 224-231.

[17] Kavoi, B.M. and Jameela, H. (2011): Comparative morphometry of the olfactory bulb, tract and stria in the human, dog and goat. Int J Morphol, 29(3): 939-946.

[18] Hall, L.W.; Clarke , K. W. and Trim, C.M. (2001): Veterinary Anaesthesia: Anaesthesia of birds, laboratory animals and wild animals. Vet. Anaesth, Saunders, London. 463, 479p.

[19] Scherle, W. (1970): A simple method for volumetry of organs in quantitative stereology. Mikroskopie, 26(1): 57-60.

[20] Suvarna, S.K, Layton, C. and Bancroft, J. D. (2019): Theory and practice of histological techniques, 8 th edition. Elsevier.

[21] Noreldin, A.E.; Elewa, Y.H.A.; Kon, Y.; Warita, K. and Hosaka, Y.Z. (2018): Immunohistochemical localization of osteoblast activating peptide in the mouse kidney. Acta histochem, 120(4): 323-328.

[22] Noreldin, A.E.; Sogabe, M.; Yamano, Y.; Uehara, M.; Mahdy, M.A.A.; Elnasharty, M.A.; Sayed-Ahmed, A.; Warita, K. and Hosaka, Y.Z. (2016): Spatial distribution of osteoblast activating peptide in the rat stomach. Acta Histochemica, 118(2): 109-117.

[23] El-Behery, E.I.; El-Naseery, N.I.; El-Ghazali, H.M.; Elewa, Y.H.A.; Mahdy, E.A.A.; El-Hady, E. and Konsowa, M.M.H. (2019): The efficacy of chronic zinc oxide nanoparticles using on testicular damage in the streptozotocin-induced diabetic rat model. Acta Histochem, 121(1): 84-93.

[24] Ibe, C.S.; Ikpegbu, E. and Nlebedum, U.C. (2018): Structure of the Main Olfactory Bulb and Immunolocalisation of Brain-Derived Neurotrophic Factor in the Olfactory Layers of the African Grasscutter (Thryonomys swinderianus-Temminck, 1827). Alex J Vet Sci, 56(1): 1-10.

[25] Shoshani, J.; Kupsky, W.J.; Marchant, G.H. (2006): Elephant brain. Part I: gross morphology, functions, comparative anatomy, and evolution. Brain Res Bull, 70(2): 124-157.

[26] Wei, Q.-G.; Zhang, H.-h. and Guo, B.-R. (2008): Histological structure difference of dog's olfactory bulb between different age and sex. Zool Res, 29(5): 537-545.

[27] Corfield, J.R.; Price, K.; Iwaniuk, A.N.; Gutiérrez-Ibáñez, C.; Birkhead, T. and Wylie, D.R. (2015): Diversity in olfactory bulb size in birds reflects allometry,

ecology, and phylogeny. Front neuroanat, 9: 102.

[28] Brunjes, P.C.; Feldman, S. and Osterberg, S.K. (2016): The Pig Olfactory Brain: A Primer. Chem Senses, 41(5): 415-425.

[29] Ngwenya, A.; Patzke, N.; Ihunwo, A.O. and Manger, P.R. (2011): Organisation and chemical neuroanatomy of the African elephant (*Loxodonta africana*) olfactory bulb. Brain Struct Funct, 216(4): 403-416.

[30] Tillet, Y.; Thibault, J. and Dubois, M.P. (1987): Immunocytochemical demonstration of the presence of catecholamine and serotonin neurons in the sheep olfactory bulb. Neuroscience, 20(3): 1011-1022.

[31] Marschner, C.(1970): Qualitative and quantitative studies on the olfactory bulb of elephants in comparison with that of man and pigs. Acta Anat , 75(4): 578-595.

[32] Shipley, M.T. and Ennis, M.(1996): Functional organization of olfactory system. J. Neurobiol, 30(1):123-176.

[33] Kosaka, K. and Kosaka, T. (2005): synaptic organization of the glomerulus in the main olfactory bulb: compartments of the glomerulus and heterogeneity of the periglomerular cells. Anat Sci Int, 80(2): 80-90.

[34] Bang, B. and Wenzel, B. (1985): Nasal cavity and olfactory system. In AS King, J McLelland. Form and Function in Birds,

3rd Edition. Academic Press, London. 195-225.

[35] Lee, T.K.; Park, J.H.; Ahn, J.H.; Park, Y.E.; Park, C.W.; Lee, J.C.; Choi, J.H.; Hwang, I.K.; Kim, S.; Shim, J.; Go, S.; Lee, E.; Seo, K. and Won, M.H. (2019): Parvalbumin-immunoreactive cells in the olfactory bulb of the pigeon: Comparison with the rat. Anat Histol Embryol, 48(4): 334-339.

[36] Steiger, S.S.; Kuryshev, V.Y.; Stensmyr, M.C.; Kempenaers, B. and Mueller, J.C. (2009): A comparison of reptilian and avian olfactory receptor gene repertoires: species-specific expansion of group gamma genes in birds. BMC Genomics,10: 446.

[37] Hamilton, K.A.; Heinbockel, T.; Ennis, M.; Szabo, G.; Erdelyi, F. and Hayar, A. (2005): Properties of external plexiform layer interneurons in mouse olfactory bulb slices. Neuroscience, 133(3):819-829.

[38] Mori, K.; Kishi, K. and Ojima, H. (1983): Distribution of dendrites of mitral, displaced mitral, tufted, and granule cells in the rabbit olfactory bulb. J Comp Neurol, 219(3): 339-355.

[39] Lahr, G.; Heiss, C.; Mayerhofer, A.; Schilling, K.; Parmer, R.J.; O'Connor, D.T. and Gratzl, M. (1990): Chromogranin A in the olfactory system of the rat. Neuroscience, 39(3): 605-611.

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الملخص العربي

الاختلافات التركيبيه للبصلات الشمية الرئيسية في إانواع محددة من الكلاب والماعز

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السمة التركيبيـة المميزةللبصلـة الشمية الرئيسية تختلـف مـن فصيلـة لاخري ومـع ذلك ، فـإن العلاقـة بـين هذه البنية والوظيفة الشمية وكذلك السلوكيات الإيكولوجية والتطورية أقل فهمًا. لذلك ، كـان الهدف مـن الدراسـة هو توضيح الفروق بـين أنواع الحيوانات في بنية البصلة الشمية في نوعين مختلفين بيئيًا مثل الكلاب والماعز، تم الحصول على ثلاثة عشر رأسًا مـن كل نوع. ثم تم فصـل المخ والبصيلات الشمية من أجل فحصها بـالعين المجردة (تشريحيا) ، وبـالمجهر الضوئي هستولوجيا ومناعيا للكشف عن البروتين الحمضي الليفي الدبق والكروموجرينين أ . فقد أظهرت النتـائج تشريحيا ، كـان حجم البصلـة الشمية بالنسبة إلي حجم المخ بأكمله أعلـي في الكلاب (١.٧٤ ٪) من الماعز (٠.٥٧ ٪). وهستولوجيا تم تنظيم البصلة الشمية الرئيسية في الطبقات التالية: طبقة العصب الشمي ، الطبقـة الكبيبيـة ، طبقـة الضفيرة الخارجيـة، طبقـة الخليـة التاجيـة ، طبقـة الضـفيرة الداخليـة ، طبقـة الخلايـا الحبيبيـة وطبقـة حـول البطين، وكانت فصيلة الكلاب تتميز بأعداد كبيرة مـن الخلايا التاجية(المترال) والخلايا المجاورة للكبيبة (الجاكستاجلومريولار) ، وسماكة الطبقة الكبيبية (الجلومريولار)، وطبقة الضفيرة الخارجية ، وطبقـة الخلايـا الحبيبيةعنها مـن تلك الموجودة في الماعز. ومناعيـا، كانت نسبة مسـاحة بـروتين الجي فـاب و السينابتوفيسين و الكروموجرانين أعلى بكثير في الكلاب عن الماعز. وكان متوسط عدد الخلايـا العصبية للخلايا التاجية فـي منطقة قياسية أعلـى بكثير في الكلاب (٣٧ ± ٦) أكثر من الماعز (٣.١٦ ± ٣١). وأخيرًا ، قد تكون هذه الاختلافات الواضحة بين الكلاب والماعز مؤشراً على تفوق االحساسية الشمية وبالتالي السلوكيات البيئية ونمط الحياة في الكلاب.

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