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Original Research

Comparative Anatomy and Functioning of Olfactory Tract in Selected Canidae

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Abstract

The sense of smell is the main sensation in wild animals necessary for hunting, and survival. A good example of such animal is grey wolf, which is able to sense the victim from a distance of up to 2 km. Domestic dogs, especially brachycephalic, because of the morphology of nasal cavity and life's conditions lost the so high developed olfactions ability. In that work, the multidisciplinary analysis of olfactory bulb responsible for olfaction were carried out. The research of macro- and microscope analyses, and also the technique of flow cytometry were used to estimate the level of high and low activity glomeruli in olfactory bulb in investigated animals. It was observed that in grey wolf the level of active glomeruli is higher than in badger and fox and the olfactory bulb has a higher size. That observations command that olfactory bulb as a part of telencephalon has the ability to create a new synapse depending on living conditions of animal.

Keywords: olfactory bulb, olfactory abilities, flow cytometry, sensation

Introduction

Sensation is constructed through many dynamic sampling of the whole external world, as exemplified by the saccadic eye movements that underlie visual perception, or active touch in somatosensation. Then the stimuli of nature are available after being received by the sensory receptors, act on nerve impulses and are delivered to encephalon. In olfaction, sensory impressions have the form of chemical stimuli (odorants) and to a large extent they are regulated by respiration behavior. Animals exhibit a rich repertoire of olfactory sampling behavior directly depending on behavioral context [1]. The ability to detect, analyze, and exploit odors appears to reach its highest degree of development in mammals [2]. An important element affecting the sensitivity of smell is the way of life and obtaining food. Therefore, it is likely that wild animals differ significantly in olfactory sensitivity from humans and domestic animals.

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The Use of Canine Smell Sense

Very different olfactory abilities are shown by dogs of different breeds. Dogs were used as a chemical detectors already 12 000 years ago simultaneously with their use during hunting. Canine smell played very important role also during Word War II when dogs were been used extensively by the military to locate explosives [3]. Today civilian used dogs to detect among other guns, bombs, flammable and ignitable liquid residues, drugs, gold ore, contraband food, gypsy moth larvae, pipeline leaks, and for forensic evidence. Dogs are also used to search people from debris and snowdrifts [4].

They is some scientific evidence that the sense of smell is one the major senses used by dogs, such as documented low thresholds for detection of odors, the anatomy of the olfactory system of the dog, and observations that dogs with measured or perceived problems with the sense of smell do not perform well in detection tasks [4]. Behavioral experiments have shown that the olfactory capability of the dog for detecting certain odors is at least 100 times greater than that of human [3]. However, as a result of intensive human selection specialized breeds suitable for different tasks were produced. Especially, domestic dogs have been submitted to a vast process of artificial selection, with a focus on specific cognitive and behavioral skills relative to the function for which the dogs were used (i.e., shepherding, hunting, search rescue), and to date, there are hundreds of breeds that differ in their physical and behavioral features [5]. Due to the fact that the dog comes from a wolf, it can be regarded as an animal with the original anatomy of the sense of smell and referred to modern breeds of dogs. In the scientific literature, there are few works showing this sense in wild Canidae. Understanding the differences can contribute to a better understanding of functional anatomy and behavior of both this group of animals and people.

Olfactory Bulb – Smell Command Center

The olfactory bulb is located an outgrowth of the forebrain, specialized for processing the molecular signals that give rise to the sense of smell. It is the exclusive recipient of input from olfactory receptor neurons in the nose. The impuls received by olfactory bulb is than transmitted by mitral and tufted cells directly into higher brain centers such as piriform cortex, amygdala, and entorhinal cortex [1, 7]. Many authors described the typical cell organization circuit in olfactory bulb, however, they pointing that the occurrence of differences in cells morphology and their number in particular layers is possible. That differences cay be a key to understanding high perceptive ability in macrosmatic animals in contrast to that animals in which the smell is not the main sense.

The standard model of cells circuit of olfactory bulb consists of 6 layers: a layer of olfactory threads (axons entering from the nasal cavity), the glomerular layer in which the axons of olfactory receptor neurons enter, make synapses and terminate in the glomeruli; external plexiform layer projecting axons into the internal plexiform layer; mitral cells layer with mitral cells and tufted cells extend dendrites into the glomerular layer and next external plexiform layer; internal plexiform layer, and granule cells layer with granule cells without axons (dendrites of that cells penetrate the external plexiform layer, internal plexiform layer and granule cell layer) [8].

Olfactory Glomeruli

Olfactory glomeruli act as functional units in coding olfactory information and contain a complex network of synaptic connections [9]. The glomerular layer of the olfactory bulb forms a kind of map of olfactory axon terminals. The axonal projection of olfactory sensory neurons to the glomeruli is precisely arranged in such a way that olfactory sensory neurons expressing a given odorant receptor converge their axons to a few topographically fixed glomeruli. Since individual glomeruli represent a single odorant receptor, the glomerular layer forms a map of odorant receptors [10].

An important role in the developing and maintaining of glomeruli shape play the neuropil-associated glia cells, which form the nearly complete envelope that appears so conspicuously around each adult glomerulus [11-12].

Our attention in that work has been focused especially on glomeruli as the relay station between the first section from the nasal cavity and the subsequent cells that make up the pathways in the brain. Moreover, our aim was to compare the macro- and microanatomy of olfactory bulb in wolf and dogs as well as between selected individual dog breeds.

Materials and Methods

Animals

Studies were conducted on 5 specimens of the dog (*Canis familiaris*), 7 of the grey wolf (*Canis lupus*), 3 of the fox (*Vulpes vulpes*) and 3 of the badger (*Meles meles*). Dog individuals had been euthanized due to incurable health condition, mostly caused by a severe trauma, the rest of animals which were found dead in their natural habitat in Poland. The dogs owners agreed to collect the tissues for the experiments. Heads of all investigated animals were dissected from the rest of the body at the level of first cervical vertebra and then sagittal section were done. Olfactory bulbs were isolated and subjected to microscopic and cytometric analyzes.

Methods

Morphology

The macroscopic structure of nasal cavity of investigated animals were assessed and compared.

Scanning Electron Microscopy

In scanning electron microscopy, olfactory bulb cells were collected with a sterile swab and the smear on the surgical steel plate was done. Then, preparations were air dried and immersed in formalin for 2 hours. After it were dehydrated in ascending grades of ethanol (10% up to 99.8% ethanol). Dried tissues were mounted on metal stub, coated with platinum (16 nm thick) using sputter coater (Quarum Q150tes) and examined under a Zeiss EVO18 Scanning Electron Microscope.

Flow Cytometry with Bioscreening

The viability of cells from olfactory bulbs was evaluated using the advanced approach being a combination of cellular integrity assessment and measurement/detection of the intracellular concentration of amines (increased intracellular amines levels are associated with cell death). For this purpose the Fixable Viability Stain 660 reagent was employed. This dye due to its high affinity to intracellular amines is an alternative for the PI or SYTOX family dyes, which discriminate live and dead cells mainly on the basis of cellular membrane integrity. Those routinely used dyes penetrate easily through disrupted (compromised) membranes and has affinity to genomic DNA. The Fixable Viability Stain 660 dye has the potential to detect dead cells as it stains additional marker of cell death – intracellular amines. This enables live/dead discrimination, to some extent, irrespectively of the integrity status of cell membranes. Moreover, as the stained cells were measured using the imaging flow cytometer we were able to discriminate olfactory bulb cells from non-cellular debris, cellular debris and cellular aggregates based on the brightfield digital image processing parameters like Aspect Ratio and Area to characterize shape and size of the analysed objects. As a result of the excitation (maximum = 649 nm), the dye emits red fluorescence (maximum = 660 nm), whose intensity is a measure of changes in cell viability (necrotic cells are characterized by the highest level of intensity of red fluorescence). Analysis of the prepared samples was carried out using a flow cytometer with FlowSight imaging (Luminex , USA) equipped with 3 lasers (405 nm, 488 nm and 625 nm), 5 fluorescence channels (acquisition in the form of a multi-channel CCD camera) and a scattered laser detector - side scatter (SSC). The characteristics of the cells were carried out based on the following parameters : aspect ratio and area, derived from digital image processing of cells in a bright field of view, raw max pixels and intensity from the SSC detector (channel 6), and intensity of red fluorescence (channel 11) excited by red laser (642 nm) derived from the Fixable Viability Stain reagent 660. Data were analyzed using Ideas software (Amnis Merck Millipore). Cell sub-populations were defined by gating on a dot plot of the relationship of red fluorescence (channel 11) to the area of the signals from the clear field of view (channel 1).

Results

Microscope Analyzes

Olfactory bulb of all investigated Canidae were observed in light microscope. In all probes the olfactory glomeruli were observed. The diameter of glomeruli were significantly different in even inter individual dog`s breeds. Generally, glomeruli in investigated Canidae can be divided into 3 groups dependently on their diameter: small (75-125 μm), medium (200-300 μm) and big (575-624 μm). All 3 groups of glomeruli were observed in olfactory bulb in all investigated animals (Fig. 1).

Flow Cytometry with Bioscreening

As it was mentioned above, glomeruli are a very metabolically active nervous structure. So, it was

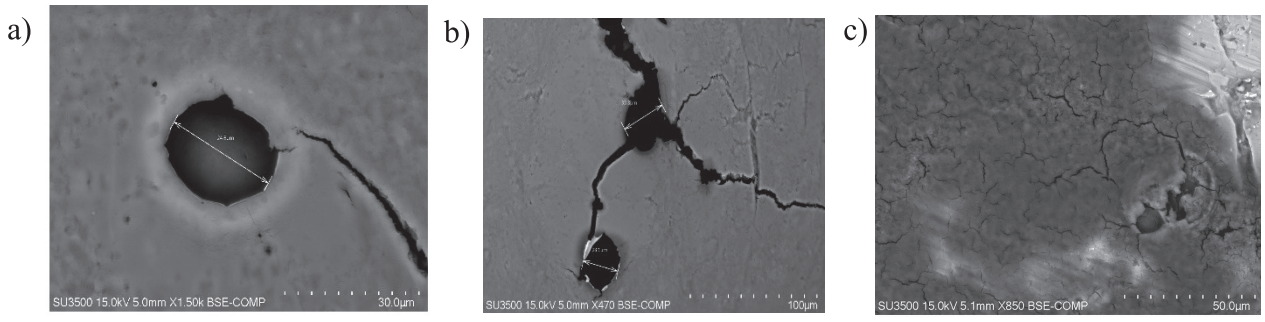

Fig. 1. An examples of glomeruli in olfactory bulb in investigated animals. a) *Canis lupus,* b*) Canis familiaris* (Syberian Husky), c) *Meles meles.*

decided to estimate intracellular metabolic activity of glomeruli in olfactory bulb of investigated animals. Glomeruli were divided into 3 groups: active, low active, and dead (Fig. 2). As we can see, tested Canidae individuals differed in the percentage of glomeruli in each group. The sensitivity of olfaction is directly connected with the number of active glomeruli. It is one of the most important factors informing us about the olfaction ability in tested animals.

Moreover, the shape and size of dominant cells were estimated (Table 1). It was observed that size of glomeruli and their metabolic activity are correlated – the smallest glomeruli are highly active, medium have decreased activity and the biggest are death. Moreover, the smallest have more regular shape, and the biggest are unregular. Such observations were compatible for all tested individuals. It confirmed our theory, developed during hours of observation of microscopic specimen of olfactory bulb of divers animals, that glomeruli arise as small structures and grow over time and eventually die.

Discussion

Morphology

Olfactory bulb is located in in the nasal part of the forebrain and has divers size and shape in investigated animals. Patterns of flow during inhalation in Canidae show that most air passes ventrally through the nasal airway, en route to the nasopharyngeal duct. Air that enters the naris dorsally tends to flow via a dorsal conduit to the rear of the nasal cavity where most of the olfactory epithelium is located. Once air from the dorsal conduit reaches the epithelium region, it slows down substantially. It migrates ventrally and laterally before passing over the transverse lamina and then exiting the airway at the choana, along with the rest of the non-olfactory inhaled air [13-16].

The size of glomeruli is diverse in individual species of animals. Our observations showed that in investigated dogs the medium glomeruli were dominant.

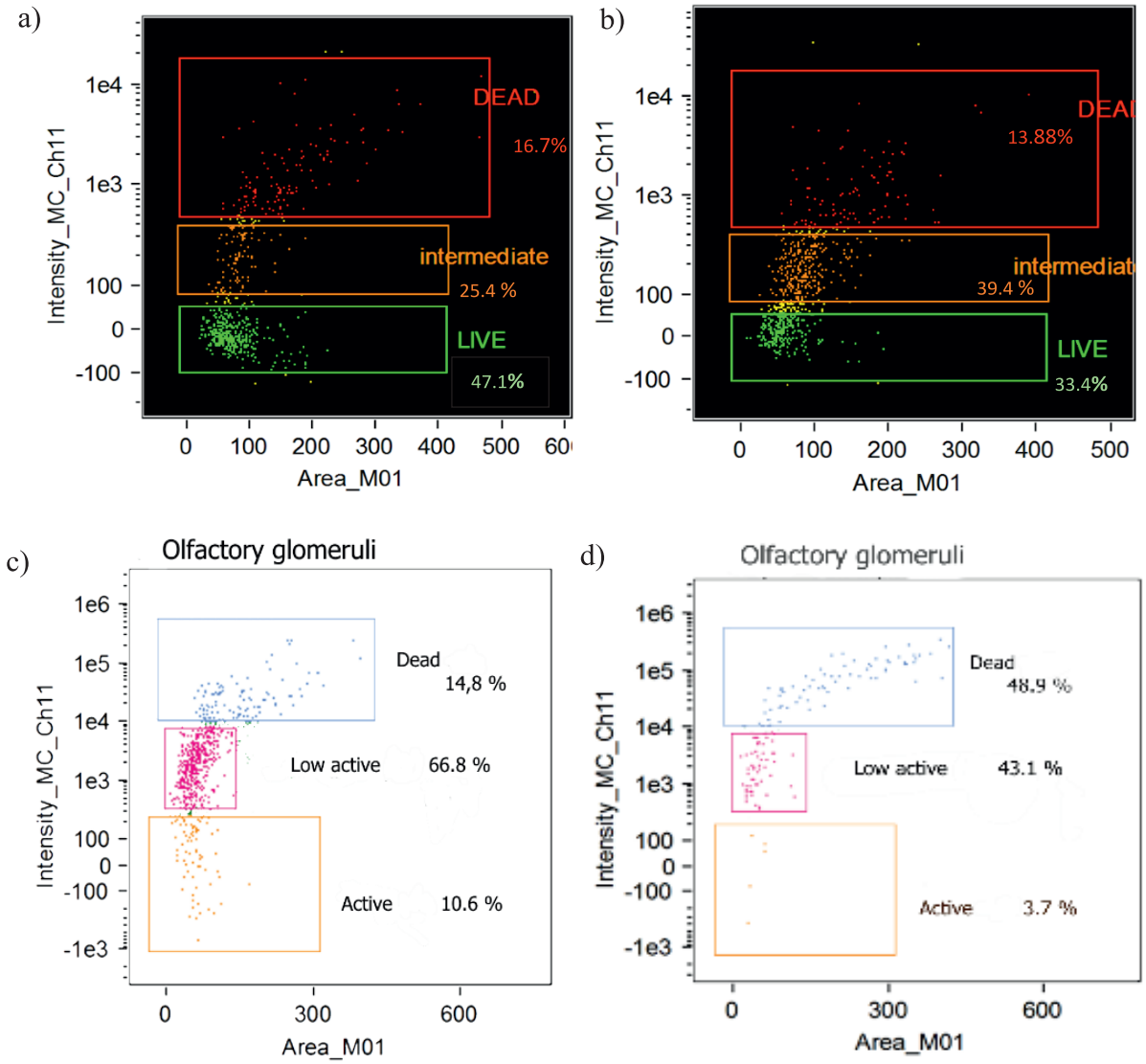

Fig. 2. Glomeruli in 3 groups a) *Canis lupus* b) *Canis familiaris* c) *Vulpes vulpes* d) *Meles meles.*

Table 1. The dominant morphology and glomeruli activity in investigated Animals.

Canidae	Glomeruli activity	Dominant morphology
Domestic dog Syberian Husky	Dead cells	
	Low active cells	
	Active cells	
Domestic dog German Shepherd	Dead cells	
	Low active cells	
	Active cells	
Grey wolf	Dead cells	
	Low active cells	
	Active cells	
Fox	Dead cells	
	Low active cells	
	Active cells	

Table 1. Continued.

Badger	Dead cells	
	Low active cells	
	Active cells	

That observation is not in line with the investigations presented by Leise [17-20] which stated that olfactory glomeruli in mammals have diameter equal between 50 and 200 μm. However, our observations are in accordance with Galizia and Menzel [21] that glomeruli are located in each animal in some specific individual maps. Mori et al. [10] stated that glomeruli can be divided into clusters dependently on their localization in olfactory bulb and recognized odorants. That are: cluster A located at the anteromedial part of the dorsal surface of olfactory bulb, respond to aliphatic acids and aliphatic aldehydes; cluster B located in the anterior region of the lateral part of the dorsal surface of olfactory bulb, respond to aliphatic alcohols with a relatively long carbon chain and to a wide range of aliphatic ketones, as well as esters; cluster C located at the central region of the lateral part of the dorsal surface, respond to salicylaldehyde, phenols and phenyl ethers; cluster D located at the caudal region of the lateral part of the dorsal surface, respond to wide structural classes of odorants like ketones (aliphatic ketones, aliphatic-aromatic ketones, diketones, and cyclic ketones); cluster E located at the dorsal-most part of the lateral surface, did not systematically respond to any classes of odorants tested by authors; and cluster F located at the rostroventral part of the dorsolateral surface, respond to aliphatic ketones, secondary alcohols, phenyl ethers, diketones, aliphatic-aromatic ketones, and cyclic ketones; cluster G located at the ventrocaudal part of the dorsolateral surface, respond to phenyl ethers, diketones, aliphatic ketones with relatively short side chains, aliphatic-aromatic ketones, cyclic ketones, and ethers; cluster H extend from the posterocentral region to the posterodorsal region of the postero-ventrolateral surface respond to one or more of the benzene-family odorants; cluster I located at the anteroventral region of the postero-ventrolateral surface respond to at least one of the cyclic terpene hydrocarbons [10, 22-25]. Unfortunately, the authors did not calculate the diameter of glomeruli in particular clusters.

Flow Cytometry with Bioscreening

According to Malun et al. [22-27] glomerular organization of the olfactory bulb is initiated by the arrival of sensory axons from the antenna. Bundles of axon terminals coalesce into spheroidal knots of neuropil assigned as protoglomeruli. Previous studies have suggested that the protoglomeruli form a template for the mature glomerular array, but an early role for projection neurons in establishing the template has not been excluded. Protoglomeruli form in a wave beginning at the entry point of the antennal nerve and proceeding across the lobe to the opposite pole. A second wave follows in which projection neurons become tufted and innervate the newly formed glomeruli, sometimes extending into the glial border surrounding the protoglomeruli. The early presence of projection neuron processes in the protoglomeruli and the formation of at least one glomerulus-like structure in unafferented lobes suggest that uniglomerular projection neurons play an active role in the construction of olfactory glomeruli [22-23, 27-32]. The formation of mature glomeruli is a highly metabolically active process, as well as maturation of glomeruli. That's probably the reason why the youngest (and simultaneously the smallest) glomeruli are the highest activity ones. Unfortunately, we cannot compare our observations with other studies, because these are the first scientific studies focusing on the analysis of glomeruli for their metabolic activity using flow cytometry. However, we are sure that these preliminary studies open up a very important research path related to learning about olfactory in mammals.

Our cytometry analyses give an important information about the tenderness for the sense of smell. Additionally, these are the first study using flow cytometry to determine the activity of the nerve structures associated with olfaction.

Conclusions

The process of domestication influenced for the evolution of olfaction. Wolf as a wild animal needs a very special olfactory abilities for hunting. Dog in the domestic conditions lost the highly developed olfaction.

Author contribution

Conceptualization K.S-L.; Methodology and investigation K.S-L, W.J, T.U.; Writing K.S-L, T.U; Supervision K.S.L., T.U.

Conflict of Interest

The authors declare no conflict of interest.

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