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The dog vomeronasal organ: a review

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Abstract: The vomeronasal organ (VNO) or Jacobson's organ is an auxiliary olfactory sense organ in many vertebrates. This organ is involved in the Flehmen response and it is necessary for the detection of pheromones, chemical signals released to members of the same species for social and sexual communication.

In this review the Authors report on the VNO in dog pointing out the morphogenesis, the structure and the neural pathway toward dog accessory olfactory bulb (AOB). By recent immunohistochemical studies it is highlighted that dog VNO neurons express only VR1 genes in their genome, unlike the rodents where VR1 and VR2 genes are expressed. Besides, the organization of AOB is lower than that of the rodents. This feature might be the result of VNO regressive process of a macrosmatic mammal, like dog.

Key Words: dog; morphofunctional study; vomeronasal organ.

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Introduction

The vomeronasal organ (VNO) consists of a pair of blind, tubular, mucomembranous ducts of olfactory epithelium lying on each side of the rostral base of the nasal septum. It is surrounded by a thin plate of cartilage and opens rostrally into the incisive duct of the same side that connects the nasal and oral cavity.

VNO is considered a second olfactory organ involved in parental, social and sexual communication as a chemosensory organ which has the chemoreceptors necessary for the detection of pheromones, chemical signals released into the environment to members of the same species and of flavor of food. The VNO is also related to 'Flehmen reaction', a peculiar retraction of upper lip (Ylmaz et al., 2007).

VNO neurons are closely related to the hypothalamus and participate in its activation via the accessory olfactory bulb (AOB) and the amygdala (Keverne, 1999).

Fig. 1. Dog nasal septum (lateral view), sensory (yellow-brown) and respiratory (red-orange) mucosa, VNO localization (panel). From Barrios et al., 2014b.

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VNO in the animal kingdom

VNO was first described by Jacobson (1813) in mammals nose: he assessed that the VNO was a sensory and secretory organ.

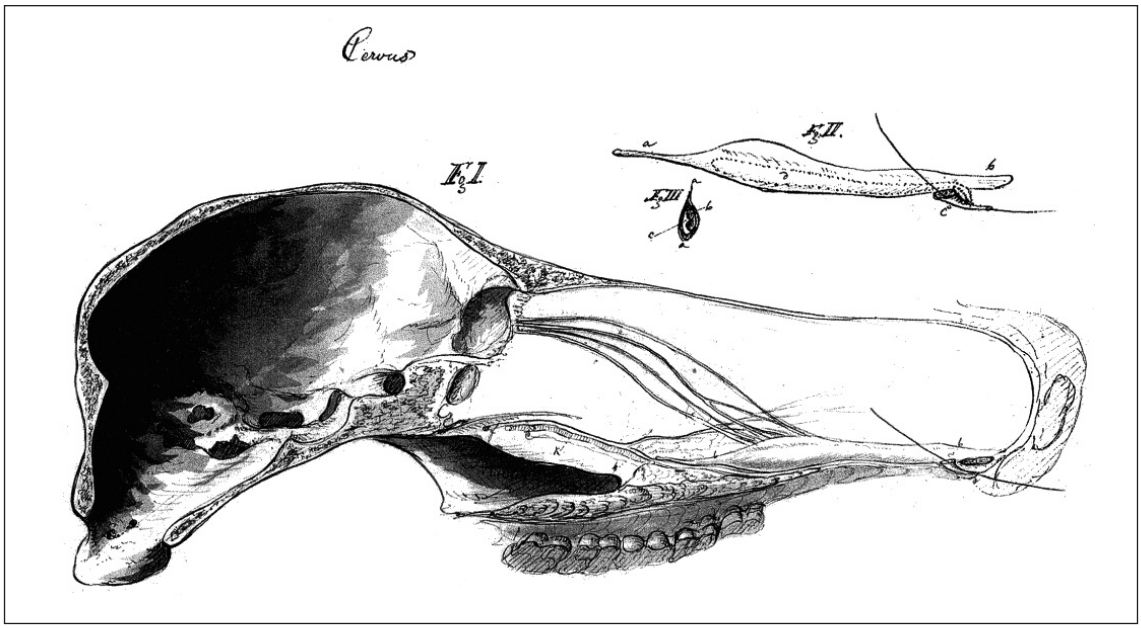

Fig. 2. Jacobson's drawing of the deer head (Cervus sp.). From Døving & Trotier, 1998.

Fig. 2 is a reproduction of Jacobson's drawing of the head of a deer (*Cervus* sp.), showing the localization of VNO with its nerves running to the olfactory bulb. Jacobson also pointed out that the VNO diameter became greater cranio-caudally and that many glands were localized in the dorsal and ventral VNO mucosa, with small secretory ducts ending in the lumen of the organ. Jacobson described the VNO in many domesticated animals (cat, cow, dog, goat, horse, pig and sheep), and not domesticate, (tiger, camel, buffalo, deer and seal) but not in birds (Jones & Roper, 1997) although it is present in the embryo at a very early stage of development. The presence of VNO was confirmed in most mammals (Von Mihalkovics, 1899; Pearlman, 1934) and it is well developed in some primates (*Nycticebus tardigradus and the Cebus capucinus*) but reduced or absent in *Macaca mulatta* (Jordan, 1972; Stark, 1975). Retzius (1894) demonstrated the chemosensory function of the organ in reptiles (snake embryo) for the similarity in morphology between the olfactory neurons and VNO neurons running to the accessory olfactory bulb. Von Mihalkovics (1899) evidenced that the sensory ciliated cells were on the medial concave surface of the VNO cavity while the lateral convex surface is lined with no sensory cells. He also described a cavernous tissue, or "*eminentia fungiformis*", under the mucosa.

VNO development: morphogenesis

With regard to the VNO development (Garrosa et al., 1998), the VNO originates from the medial wall of the olfactory pit (VNO placode) and consists of a cellular bud growing dorsally, caudally and medially that forms the VNO groove. During morphogenesis the VNO groove closes forming a blind-ended tube laterally the nasal septum, which opens into the oral cavity by the

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incisive duct in dog. In cross section the VNO shows a shape similar to a kidney and the epithelium on the concave side, in a medial position, become wider and neuroblasts, supporting and undifferentiated cells develop. The epithelium on the convex side ("non-sensory" epithelium), is in a lateral position and become thinner. During late morphogenesis, VNO acquires adult shape and at birth, medial and lateral epithelia becomes similar to the adult. Histochemical and ultrastructural features suggest that full performance of the VNO does not occur at birth but in prepubertal ages.

VNO histology

The structure of the dog VNO has been reported in several studies (Barone et al., 1966; Klein, 1881; Ramser, 1935), where VNO is described as reduced, containing no true olfactory receptors (Barone et al., 1966) and histologically and ultrastructurally similar to that of the cat, guinea-pig, mouse and rabbit (Ciges et al., 1977).

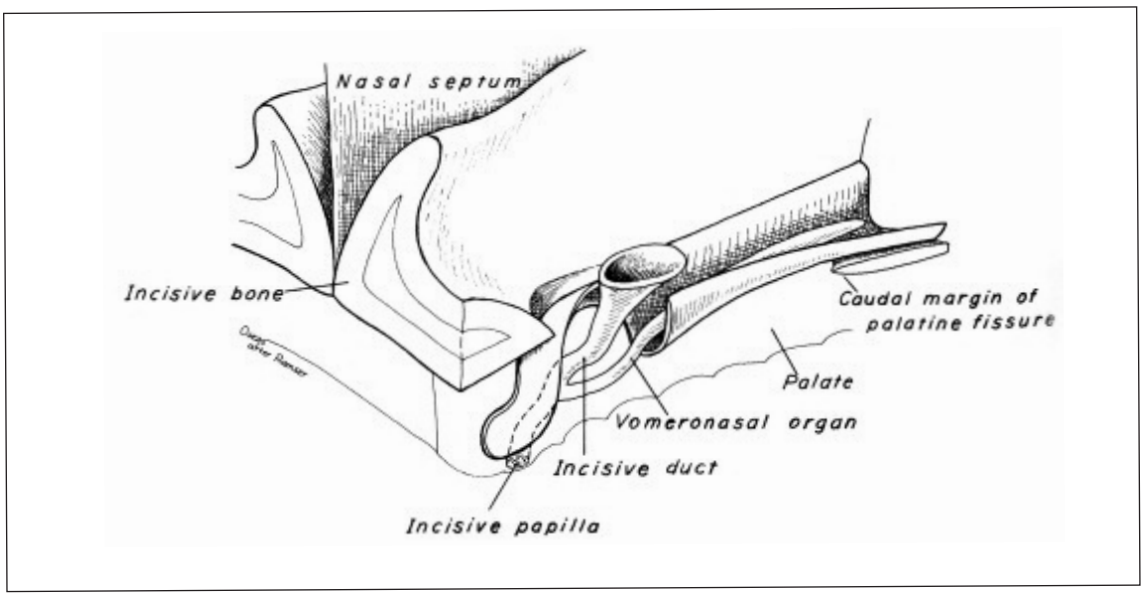

Fig. 3. Dog VNO with incisive duct. From Evans: Miller's Anatomy of the dog, Saunders, Elsevier, 1993.

Adams & Wiekamp (1984) prove that dog VNO is highly developed. They study the VNO in mature dogs with the optical, transmission electron and scanning electron microscopes. The authors report a crescent-shaped lumen adjacent to the nasal septum and enclosed partially by a J-shaped vomeronasal cartilage, lacking in the dorsal lateral wall. In cross section dog VNO lumen shows a lateral convexity along most of its length and a concavity medially in anterior portions and ventrally in caudal portions.

Both convex and concave surfaces of the epithelium show a well vascularized lamina propria. Small nerves deep to the medial epithelium are unmyelinated while those deep to the lateral epithelium usually contains some myelinated fibers. Dorsal and lateral to the VNO glands present ducts opened into the lumen through the epithelial commissures and the lateral epithelium, less frequently through the medial epithelium. The lumen is filled with fluid from the VNO glands.

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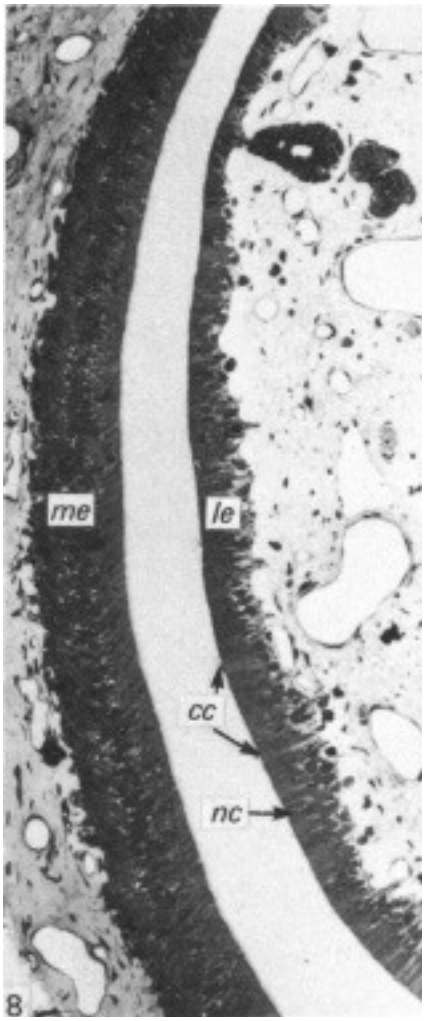

Fig. 4. Dog VNO (transverse section, caudal portion). Lateral epithelium (le) with few ciliated columnar cells (cc) and non-ciliated columnar cells (nc). Medial epithelium (me). From Adams & Wiekamp, 1984.

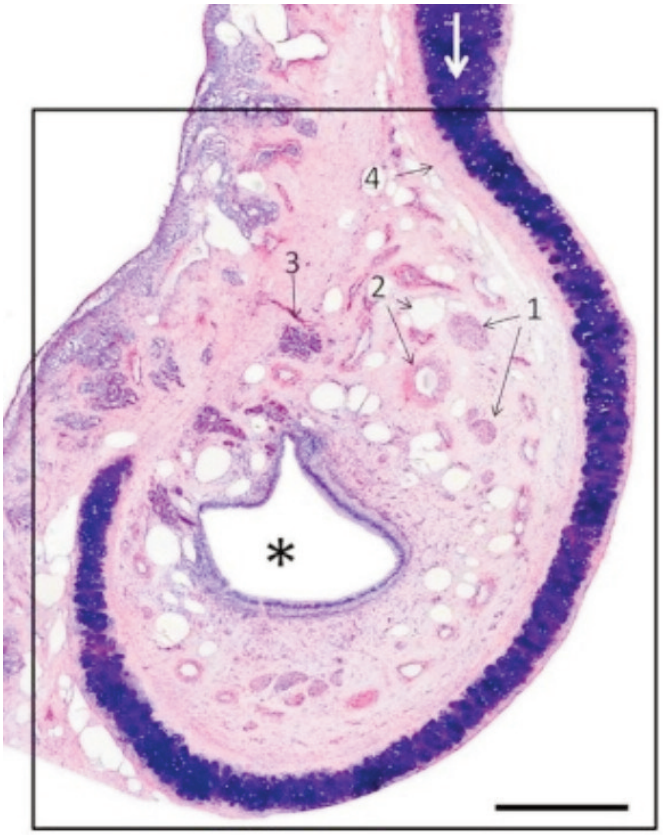

Fig. 5. Dog VNO (transverse section); left is lateral, up is dorsal. Lumen (asterisk); nerves (1); vessels (2); gland (3); connective tissue (4); cartilage (whitearrow). E/E. Scale bar: 500µm. From Barrios et al., 2014.

As reported by Adams & Wiekamp (1984), the epithelium of the lateral convex mucosa, named Non-Sensorial Epithelium (NSE) (Barrios et al., 2014b) is pseudostratified contained basal, non-ciliated columnar, ciliated columnar and goblet cells. Ciliated cells had a like-dome apex with numerous cilia and long basal bodies. The non-ciliated cells have a surface border of microvilli and are the most represented. The cilia irregularly distributed might favor the mixing of fluid in VNO lumen so that the contact between molecules and receptor cells is enhanced.

As reported by Keverne (1999), the epithelium of the medial concave mucosa, named Sensorial Epithelium (SE) (Barrios et al., 2014b) is pseudostratified containing basal or stem cells along the basal membrane, supporting cells lying in superficial layer of the epithelium, and receptor cells. This epithelium extends far caudally, unlike rat (Vaccarezza et al., 1981) and hamster (Taniguchi & Mochizuki, 1982). The receptor neurons show apical microvilli, unlike the presence of only one cilium on the surface of other mammalian VNO receptors (Ciges et al., 1977; Vaccarezza et al., 1981; Salazar et al., 1984).

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Sano and Okano (1995) divide dog VNO in a vestibular, rostral, body and caudal portion, with SE only in the medial wall of the body portion, which is comprised between the level of the canine teeth and the edge of the third premolar teeth.

In mammals, by in situ hybridization, two families of G protein-linked receptors (VR1 and VR2) are expressed in a distinct region of the VNO; each neuron expresses only one receptor (Dulac & Axel, 1995). Barrios et al. (2014) point out that in the dog VNO only VR1 is expressed.

The neural patways: from the VNO to the brain

The neural pathways from the VNO mucosa to the brain are distinct from those of normal olfactory mucosa (Scalia & Winans, 1975). This pathway projects to an accessory olfactory bulb (AOB) (Salazar et al., 1992) by two or three nerves branches merging before the ethmoid bone. The nerves penetrate the lamina cribosa by a single orifice to run along the media part of the olfactory bulb. Dog AOB position and morphology remained vague for a long time (Cajal, 1902; Miodonoski, 1968). Salazar et al. (1992 and 1994) show that dog AOB has a lower organization compared to that of the rodents; it presents a thick glomerular layer and thinner layer of mitral/tufted granular and scattered glial cells. For this reason the dog AOB has a very simple structure with reduced lamination; in particular plexiform and mitral/tufted layers are indistinguishable. Nakajima et al. (1998) by an immuno-histochemical study demonstrate the distribution of neurons in dog AOB: they point out that the granular cells are present but in small number in respect to other mammals. Because the granular cells are inhibitory interneurons in the olfactory pathway, some differences of the AOB function between dog and other animals are conceivable.

The mammalian olfactory system shows differences among mammals; in particular in rodents are detected four different nasal areas: main olfactory epithelium (MOE), septal organ (SO), Grüneberg's ganglion (GG) and vomeronasal organ (VNO). Barrios et al. (2014b) point out that dog olfactory system lacks of the SO and the GG, besides the absence of VR2 genes in the canine genome. All these features might be the result of a regressive process of the dog olfactory system (Salazar et al., 1984), despite that dog is a considered a macrosmatic mammal for its particular sense of smell. The larger size of the canine nasal cavity might allow a better physical discrimination among odors than in the mouse (Schoenfeld & Cleland, 2005).

Another possible explanation of the dog VNO regression might be related to its domestication dating back to 15.000 years ago because a smaller range of olfactory stimuli than undomesticated animals like rodents are required (Leonard et al., 2002; Savolainen et al., 2002). Also Salazar et al. (1984) assumes that the more domesticate the specie the smaller the functional importance of the VNO. Confirming this hypothesis Shi & Zhang (2008) and Niimura (2012) report that dog has 811 functional olfactory receptor genes instead the mouse that has 1035 functional olfactory receptor genes.

The Authors agree with Barrios et al. (2014b) that a study of wolf VNO would significant to determine whether the dog VNO might be a result of domestication.

Unresolved issues and future directions

Actually there are many evidences that the main olfactory system is involved also in pheromonal communication. Indeed, previous works considered the "dual olfactory hypothesis", sustaining that the main and accessory olfactory bulb project in non overlapping areas of the brain in the basal telencephalon. According to this hypothesis the olfactory and vomeronasal systems were organized as anatomical pathways serving different functions. Recently (Martinez-Marcos, 2009; Tirindelli et al., 2009), reported that, by genetic studies, the olfactory and vomeronasal epithelia are able to process both olfactory cues as pheromones. A hypothesis predicts that main olfactory and

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vomeronasal pathways converge overlapping in the rostral basal telencephalon defining a new category of mixed chemosensory cortex. Other hypothesis states that the two pathways of the accessory olfactory bulb projects primary to amygdala as target and then tertiary pathways directed to ventral striatum converge to a same sensory cortex. These hypotheses indicate that anatomical and functional distinction between the olfactory and vomeronasal systems should be rewieved and they could justify that, despite the reduction of the VNO development resulting in domestication, dog should be considered a macrosmatic species.

The VNO function can also be evaluated by the studying behavioral alterations in spontaneous or experimentally induced VNO lesions. Wysocki et al. (1991) in rodents, after VNO removing, conclude that vomeronasal organ is determinant for modulating the behavior in reproductive function by the perception of pheromones. Booth et al. (2000) evidence the role of the sheep VNO in lacking of recognition of neonatal offspring by cauterization of the nasoincisive duct which makes nonfunctional this organ. Asproni et al. (2015) study the effects of inflammatory lesions of cat VNO and point out the association between inflammatory lesions with intraspecific aggressive behavior. This type of investigation could also be applied to the dogs to determine the actual involvement of the VNO in intraspecific communication and in the adaptive mechanism to new environments, as a recent study suggests (Osella et al., 2015).

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L'organo vomeronasale nel cane: una review

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Sintesi

L'organo vomeronasale (VNO) consiste di un paio di dotti tubulari, a fondo cieco, mucomembranosi, rivestiti di epitelio olfattivo che sono collocati, uno per lato, nella porzione rostrale della base del setto nasale. Il VNO è circondato da un sottile strato di cartilagine e si apre rostralmente nella cavità nasale o in quella buccale.

Il VNO è considerato un secondo organo olfattivo, coinvolto nella comunicazione in ambito parentale, sociale e sessuale, possedendo chemiorecettori necessari per il rilevamento dei feromoni.

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Il VNO fu descritto per la prima volta da Jacobson nel 1813 nel naso dei mammiferi e fu da lui ritenuto un organo con attività sensoriale e secretoria.

La presenza del VNO fu confermata nella maggior parte dei mammiferi da studi posteriori ed è ora noto come sia ben sviluppato in alcune specie di primati non umani-

Qualche decennio dopo le osservazioni di Jacobson, un altro anatomista, Retzius, lo descrisse nei rettili. Nel 1899 Von Mihalkovics evidenziò che l'epitelio ciliato era posizionato sulla superficie concava mediale dell'organo mentre la superficie laterale era rivestita di cellule senza funzioni chemiorecettoriali, al di sotto delle quali è presente un tessuto cavernoso detto "*eminentia fungiformis*".

La struttura del VNO del cane è riportato in diversi studi dove è descritto come un organo di dimensioni ridotte, non contenente veri recettori olfattivi e istologicamente simile a quello del gatto, cavia, topo e coniglio.

L'epitelio che riveste la porzione laterale, denominato Epitelio Non Sensoriale (NSE) è pseudistratificato e contiene cellule basali, colonnari non ciliate, colonnari ciliate e globose. Le cellule ciliate hanno un apice a cupola con numerose cilia e corpi basali. Le cellule non ciliate sono le più numerose e hanno una superficie cosparsa di microvilli. Le cilia, distribuite in modo irregolare, potrebbero favorire la miscelazione del fluido nel lumen del VNO in modo che il contatto tra le molecole e le cellule recettoriali sia migliorato.

L'epitelio che riveste la porzione mediale, denominato Epitelio Sensoriale, è pseudo stratificato e contiene cellule basali che sostengono le cellule più superficiali e quelle recettoriali. Questo tipo di epitelio si estende molto causalmente, a differenza di quanto avviene nel ratto e nel criceto.

Sano e Okano suddividono il VNO del cane in quattro porzioni: vestibolare, rostrale, corpo e caudale. L'epitelio sensoriale sarebbe presente nella porzione del corpo che è compresa tra il canino e l'apice del terzo premolare.

La via neurale dal VNO al cervello è distinta da quelle delle mucosa olfattiva normale. Questa via proietta al bulbo olfattivo accessorio attraverso due o tre branche nervose che emergono prima dell'osso etmoide.

Il sistema olfattivo presenta differenze notevoli tra i diversi mammiferi; in particolare nei roditori sono individuabili quattro differenti aree nasali: l'epitelio olfattivo principale, l'organo settale, il ganglio di Grüneberg e il VNO. Il sistema olfattivo del cane è privo dell'organo settale e del ganglio di Grüneberg. Tutti questi aspetti, oltre alla mancanza del gene VR2 nel genoma canino, potrebbero essere il risultato di un processo di regressione del sistema olfattivo del cane, nonostante questo animale sia considerato un mammifero macrosmatico per il suo particolare olfatto. Tuttavia la maggior dimensione della cavità nasale del cane potrebbe comunque permettere una migliore discriminazione fisica degli odori rispetto al topo.

Un'altra possibile spiegazione della regressione del VNO nel cane potrebbe essere la domesticazione a cui questo animale è stato sottoposto da almeno 15 mila anni.

Recentemente alcuni studi hanno valutato il ruolo del VNO nella comunicazione animale, osservando le alterazioni del comportamento quando tale organo sia lesionato, in modo spontaneo o sperimentale.

Nei roditori il VNO risulta indispensabile nel modulare il comportamento riproduttivo, permettendo la percezione dei feromoni sessuali. Negli ovini, il riconoscimento dell'agnello da parte della madre non avviene se si rende non funzionante il VNO attraverso la cauterizzazione del dotto naso-incisivo. Nel gatto, processi infiammatori del VNO sembrano correlati a fenomeni di aggressività.