Embryonic Developmental Study on Vomeronasal Organ of Montpellier Snake (Malpolon monspessulana)

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Abstract: The development of vomeronasal organ was studied in Montpellier snake (Malpolon monspessulana) at 1.2, 1.5, 1.9, 4, 5, 5.5, 6.5, 7 and 8 cm Snout vents lengths. The vomeronasal organ started its development as a vomeronasal placode. The latter continued in its growth (vomeronasal pit-vomeronasal sac) until reached the dome shape. The vomeronasal epithelium became differentiated into thick sensory epithelium and thin non sensory epithelium. The developing dome shape vomeronasal organ opened into the oral cavity through vomeronasal duct. Accessory nasolacrimal duct opened medially into vomeronasal duct. The sensory epithelium of the organ gave off nerve fibers which form the vomeronasal nerve. Finally, the vomeronasal organ was supported by a cartilaginous capsule and membrane bones.

Key words: Malpolon monspessulana • Vomeronasal organ • Vomeronasal duct • Accessory nasolacrimal duct • Vomeronasal nerve

INTRODUCTION

The vomeronasal or Jacobson's organ (VNO) is a pair of blind-ended cavities or tubules present in many vertebrates [1-4]. It is absent in fishes and some mammals as chiropterans, cetaceans and sirenians [5-10]. It is believed that the vomeronasal first evolved in amphibians [11-13]. Among reptiles, the vomeronasal shows a wide variation in occurrence, structure and development [14-16]. Moreover, the highest variability and diversification were found in recent reptiles among the members of order Squamata [17-18-19-20]. Chamaeleonids showed the most extreme degree of reduction of the organ [21, 22]. Snakes are mostly dependent on their smell and touch senses for detection [23], which appeared to be essential component to the snakes for a perfect accommodation of its way of life. The development of the nasal chemical senses of snakes is of particular interest since evidence suggested that the neonatal reptiles use both their olfactory and vomeronasal system [24]. Embryologically, the vomeronasal organ derived from the olfactory placode and is both morphologically and physiologically similar to the main olfactory system [15]. Although, there are many anatomical and physiological studies on the vomeronasal organ of snakes, yet there were little studies on its ontogenetic development. There were many ontogenetic studies on the vomeronasal organ of both amphibians [25, 26] and mammals [10, 27-31]. The all sense organs, the vomeronasal organ originated from the cranial placode. It arose in common with the main olfactory organ from the olfactory placode. The cranial placodes vitally contributed to the formation of the paired sense organs and to the cranial sensory ganglia. These placodes are specialized areas of the head ectoderm of the vertebrate embryos that typically first became apparent as patches of thickened columnar epithelial cells [32]. From the available reptilian literatures, it appeared that, there are many anatomical and physiological studies on this organ, but its ontogenetic studies or studies on its morphogenesis were rare or scarce. Hence, the present work aimed to study the embryonic development of this organ in the successive embryonic stages. Another aim was to compare the results as well as those of other snakes and lizards and with the other vertebrate taxa.

MATERIALS AND METHODS

The snake used in this investigation is the Montpellier snake; Khodari Malpolon monspessulana (Family: Colubridae, Suborder: Ophidia, Order: Squamata).
Table 1: The Snout vents lengths (SVL cm) of the studied stages of *Malpolon monspessulana*.

<table>
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<th>stages</th>
<th>1</th>
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<td>SVL (cm)</td>
<td>1.2</td>
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<td>1.9</td>
<td>4</td>
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According to [33], it distributed from North Africa to south western Asia. In Egypt, the Montpellier inhabits western Mediterranean coastal desert, Nile Delta, lower valley and Siwa oasis. It found in the semi-desert, sandy areas of northern Delta, around vegetated salt marshes and in cultivated land. It feeds on small mammals, lizards, frogs and birds. Variations in its color include reddish individuals.

The different developmental stages were available in the fertilized collecting eggs. The latter had been getting with the help of specialist. Embryos were carefully taken out of the shells and their snout vents lengths (SVL) were estimated. The Snout vents lengths (SVL cm) of the studied stages of *Malpolon monspessulana* are listed in Table 1.

Live healthy four embryos from each stage were immediately fixed in aqueous Bouin’s fixative for 24-48 hours according to the size of the embryos.

Large four embryos were treated with EDTA solution for decalcification. Taken time for decalcification process ranged from 30 to 40 days depending on the size of the embryo, during which the EDTA solution was changed every 4 days. This was followed by washing the embryos several times with 70% ethyl alcohol.

Embryos were treated with ascending series of ethyl alcohol and then cleared with xylene. Thereafter, the specimens were embedded in a paraffin wax. This was followed by sectioning embryos transversely at 10 microns thickness using Reichert microtome.

The sections of each specimen were mounted serially on microscopic slides and prepared for staining. The latter was carried out by haematoxylin and eosin.

The vomeronasal organ is examined in these sections. Several sections were chosen for photomicrography using Zeiss photomicroscope supplied by Canon digital camera to demonstrate twice different developmental changes of the organ and its relation to the different neighboring structures.

**RESULTS**

In *Malpolon monspessulana*, the vomeronasal organ originated from medial part of the olfactory placode after its invagination to form olfactory sac. The olfactory placode appeared as a thickened area of the ectoderm in a position lateral to the anterior part of the prosencephalon and anterior to the developing eyes, at stage 1 (SVL; snout vent length: 1.2 cm). Shortly latter on, at stage 2 (SVL: 1.5 cm), this placode invaginated forming a shallow olfactory pit which soon became deeper at stage 3 (SVL: 1.9 cm) forming an olfactory pouch and the olfactory sac.

Stage 4 (SVL: 4 cm): The medial part of the olfactory placode (at the olfactory sac stage) became more thickened forming the vomeronasal placode (Fig. 1, VO.P), which gradually sunk down forming a shallow vomeronasal pit (Fig. 2, VO.PI). In this stage (Fig. 2), the vomeronasal sensory epitheliums gave off a nerve fiber which forms the vomeronasal nerve primordium (VO.N.PR). This primordium adhered to the main olfactory nerve primordium (OL.N.PR).

Stage 5 (SVL: 5 cm): The vomeronasal pit (Fig. 3, VO.PI) became more deeper forming a tubular; vomeronasal sac (VO.SC) which lied medial to the main olfactory canal (OL.CN) near its posterior end (Fig. 4). While at its anterior end, the vomeronasal pit opened into the main olfactory canal.

Stage 6 (SVL: 5.5 cm): The vomeronasal sac opened into the olfactory canal from its medial side. The vomeronasal sac increased in size. Its lumen acquired a crescentic shape (Fig. 5). Here, the sensory epithelium of the organ was undifferentiated. The mushroom body primordium (M.B.PR) appeared along the ventral side of the organ consisting of undifferentiated mesenchymal tissue. Here (Fig. 6), the vomeronasal sac opened by a short vomeronasal duct primordium (VO.D.PR) together with the main olfactory canal by a common duct (CO.D). The latter opened into the palate by the internal naris (IN.NR), which appeared as an anteroposterior cleft in the palate lateral to the organ. The vomeronasal nerve (VO.N) appeared as few nerve bundles, which formed from the nerve arising from the dorsal, lateral and medial sensory epithelium. This nerve appeared entering the accessory olfactory bulb from its medial side (Fig. 7). The posterior part of the common duct elongated forming the nasopharyngeal duct (Fig. 5, NP.D) of the main olfactory organ.

Stage 7 (SVL: 6.5 cm): The vomeronasal organ (Fig. 8) became completely separated from the main olfactory organ (Chamber). It grew large and became dome shape structure. The epithelium lining its lumen became differentiated into a dorsal thick sensory epithelium (V.SE) and a ventral thin non sensory epithelium (V.NSE) covering the mushroom body.
Figs. 1-11: Photomicrograph (H&E) of a transverse section passing through the olfactory region of different embryonic stages.
Stage 8 (SVL: 7 cm): The developing dome shape vomeronasal organ developed a closed vomeronasal duct primordium (Fig. 9, VO.D.PR). It loosed its connection with the olfactory canal. In this stage (Fig. 10), the dorsal sensory epithelium of vomeronasal (V.SE) organ differentiated into 3 layers: an inner supporting layer (SP.LA), intermediate receptor layer (RE.LA) and an outer basal layer (B.LA). The floor of the developing organ (mushroom body) consisting of mesenchymal connective tissue, began to protrude into the lumen forming the vomeronasal concha primordium (VO.CO). This covered with a layer of non sensory epithelium which faces a crescentic organ lumen (LU).

Stage 9 (SVL: 8 cm): The vomeronasal organ (VO), lied ventral to the rostral part of the olfactory chamber. Its dorsal side was bulging and forming the dorsal dome while the ventral side invaginated into the organ forming mushroom body (Fig. 11, M.B). The latter was supported by a developing cartilaginous vomeronasal concha (VO.CO). The organ had a very narrow opening duct (Fig.11, VO.D.PR) which opened ventrally into the mouth cavity through the palate. This duct received from its medial side the nasolacrimal bone (SMX) support vomeronasal organ. An opening vomeronasal duct receives from its medial side the nasolacrimal duct primordium (NL.D.PR). Scale bars, 125µm.

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DISCUSSION

In the current study, the vomeronasal organ originated from the medial part of the olfactory placode after its invagination to form the olfactory sac. The olfactory placode made its appearance as thickened area of the ectoderm at 1.2 cm SVL (Snout vent length). Shortly latter on and at 1.5 cm SVL stage, the placode invaginated
forming an olfactory pit which soon became more deeper at 1.9 cm SVL stage forming a pouch like structure; the olfactory sac. This was the same results recorded by Parsons [34] in Cxysemyspicta; Thamnophis sp.; Oxybelis sp. and in Alligator mississippiensis. The olfactory placodes were specialized areas of the head ectoderm of the vertebrate embryos that typically first become appeared as patches of thickened columnar epithelial cells [32]. Halpern [15] mentioned that, the vomeronasal organ derived from the olfactory placode and was both morphologically and physiologically similar to the main olfactory system. Also, the evolutionary origins of the vomeronasal organ were thought to begin with tetrapods and specific receptor genes that were expressed in the vomeronasal system as compared to the olfactory system proper had been identified [35, 36]. These earlier stages in jacobson's organ development of Malpolon monspessulana were relatively similar to the reptiles; while in the later stage, the members of each species gradually attained their characteristic an adult specialization [15, 24, 34]. The obvious difference was the timing sequences [5]. Since discovered, the vomeronasal organ assumed that: it was the organ of secretory nature but suspected that it could also be sensory [37]. It was named as organ on vomeronasal Jacobsoni by Mihalkovics [38]. Today, this organ was recognized as a chemosensory organ for pheromones in mammals and jacobson's organ in reptiles. Concerning reptiles, recent studies suggested that the vomeronasal receptor neurons and not the main olfactory ones were stimulated by a non volatile substances extracted from earth worms which are the favorite food of garter snakes [39].

The vomeronasal organ anatomy shape, position, communication, organization and development vary considerably among vertebrates [40-42]. Phylogenetically, it seemed probably that the vomeronasal organ first evolved in amphibians [11-13, 43, 44]. Although, Hornung and Mozeli [45] and Trotier et al. [46] reported that in frogs, the organ couldn’t be considered a true organ as in reptiles and mammals but rather a specialized area of the wall of the inferior chamber of the nose. It attained its greatest development in reptiles but in some of them it was reduced and even absent in others [34, 47-51]. Aves and crocodilians were considered to be sister groups, owing to the absence of this organ in adult stages [12, 40, 52]. Although, there were many studies on the structure of the reptilian vomeronasal organ owing to its variability [15, 53], yet, there was few or scarce information about its ontogenetic development. Among reptiles, the vomeronasal organ was more highly developed in squamates and was largest in snakes [17-18, 54-58]. The developing vomeronasal organ of Malpolon monspessulana remained opened into the main olfactory canal at 5 and 5.5 cm SVL stages. At these stages, the organ repeated its conditions of the adult amphibians. Again, at 5.5 cm SVL stage, the organ lumen acquires a crescentic shape, which was lined with undifferentiated epithelium, with the appearance of the mushroom body primordium. A mushroom body was not developed in Chamaeleon and in the other lizard in which jacobson's organ was reduced [21, 22, 59], although the organ developed normally in early stages. On the other hand, in crocodilian, the organ was transitory and disappeared in later embryonic stages [34]. At 6.5 cm SVL, the vomeronasal organ became completely separated from the main olfactory system and at 7 cm SVL; it started to develop a solid vomeronasal duct into the palate. This duct hollowed out in 8 cm SVL stage to open into the mouth cavity. The disappearance of the connection with the olfactory organ and the appearance of the organ duct that opened into the mouth is atypical adult reptilian character. The epithelium lining the organ started to differentiate into a thick sensory layer on the dorsal side and a thin non sensory epithelium covering the mushroom body at 5.5 cm SVL. This was the same as that mentioned by Taniguchi et al. [60]. The sensory epithelium in stage 7, 8 cm SVL developed into tightly packed polygonal columns interspersed with small amount of connective tissue containing a network of capillaries. These results were mentioned by Wang and Halpern [61] in garter snake. Parsons [34] recorded that, these columns developed only in later embryonic stages. Such columns are not formed clearly in lizards [50, 51]. Again, the capillaries were not present at the earliest stages in the development of the columns of lizards, but they did seem to be there at all stages in Thammopis [34].

CONCLUSION

The vomeronasal organ shows the same anatomy and histology as found in other snakes. According to the importance of vomeronasal organ to reptiles and especially to Squamata further studies on vomeronasal organ are required.

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REFERENCES


