ANATOMICAL AND HISTOLOGICAL ORGANIZATION OF THE OLFACTORY SYSTEM OF FROG *MICROHYLA ORNATA*

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Abstract: Olfactory system is a sensory and it consists of two distinct parts, a main olfactory system and a vomeronasal or accessory olfactory system in amphibians. The longitudinal sections through olfactory system of adult frog *M. ornata* showed the main olfactory chamber and vomeronasal organ. The main olfactory chamber is further divided in to medial diverticulum and lateral diverticulum. The olfactory nerves originated from olfactory epithelium and terminated in to main olfactory bulb. The main olfactory bulb was divided in to three layers, glomerular layer, mitral cell layer and internal granular layer. Vomeronasal organ was seen to be bean shape and consisting of vomeronasal cavity and vomeronasal epithelium. Vomeronasal nerve originated from the vomeronasal organ and innervated to accessory olfactory bulb. The left and right part of olfactory system is separated by nasal septum. Olfactory system is an ancient chemosensory system and organization of this chemosensory system has been phylogenetically conserved.

Key words: Olfactory system, Vomeronasal organ, Amphibia

Introduction: Olfactory system is a dynamic and primitive chemosensory system which provides environmental cues to the organism. Olfactory system is an important component of the sensory system. It plays a crucial role in physiology, behavior as well as reproduction (Halpern, 1987; Eisthen, 1997; Bhatnagar and Reid, 1996; Kelliher et al., 2001; Halpern and Martinez-Marcos, 2003). All vertebrates have olfactory system and it is first developed in the fishes. In amphibians, two distinct parts of their olfactory system is: a main olfactory system and a vomeronasal or accessory olfactory system. They are anatomically and functionally different in vertebrates (Baxi et al., 2006; Reiss and Eisthen, 2007).

The olfactory system has been studied in several amphibians such as apoda, urodela and anura (Khalil, 1978; Billo and Wake, 1987; Eisthen et al., 1994; Dawley and Crowder, 1995; Eisthen,2000; Taniguchi et al., 2008). In the anurans, olfactory system is subdivided into two divisions i.e. the main olfactory chamber and vomeronasal organ. The main olfactory chamber is further divided in to lateral diverticulum and medial diverticulum and these subdivisions may lead to the dual mode of habitat in amphibians (Bertmar, 1981; Halpern, 1987; Hoffmann and Meyer, 1991). The main olfactory system consists of the olfactory epithelium and the main olfactory bulb, and second vomeronasal system includes vomeronasal epithelium and accessory olfactory bulb. The olfactory nerve originates from olfactory epithelium and terminates in the main olfactory bulb whereas vomeronasal nerves originate from vomeronasal organ and penetrates in to the accessory olfactory bulb (Scalia and Winans, 1975). The main olfactory system detects volatile, airborne substances and is involved in detection of prey and enemy while the accessory olfactory system senses fluid-phase stimuli and serves for intra-specific communication (Dawley, 1984; Houck and Reagan, 1990).

Olfactory system is well studied in the frog, *Xenopus laevis* largely because of its extensive use as model organism in laboratory research. The frog *X. laevis* possesses three main nasal cavities (Tinsley et al., 1996), principal cavity (medial diverticulum), middle cavity or (lateral diverticulum) and inferior cavity which consist of vomeronasal organ (Saint and Zylberberg, 1992; Hansen et al., 1998). In the *X. laevis,* middle cavity contains sensory epithelium while in typical terrestrial frogs middle cavity is non sensory. In the *X. laevis*, Bowman's glands have been reported in the principal cavity, but absent in the middle cavity (Millery et al., 2005). Receptor neurons of the principal cavity are ciliated, and supporting cells are secretory in nature whereas the middle cavity contains both microvillar and ciliated receptor cells and both secretory and ciliated supporting cells (Hansen et al., 1998; Oikawa et al., 1998).

In spite of many studies on the olfactory system in amphibians, no concrete information is available on the olfactory system of *Microhyla ornata*. In addition, histological features of the olfactory system may be differing among amphibian families (Key and Giorgi 1986; Saito and Taniguchi 2000), because of their habitats, behavior and genetic heterogeneity. Therefore, present study is undertaken to investigate the histological organization of olfactory system and vomeronasal organs of frog *M. ornata.*

Materials and Methods: Frog *M. ornata* were collected and fed with termites daily. Frogs were maintained in aquarium containing moist thin layer of soil on bottom. Three animals were anesthetized, olfactory system along with fore brain is dissected out and fixed in Bouin's fixative for 24 hours. Olfactory system along with the forebrain was then preserved in 70% alcohol. Tissues were dehydrated through ascending grades of alcohol, cleared in xylene, infiltrated in molten paraffin wax and embedded and blocks were prepared. Tissues were sectioned in longitudinal and transverse plane at 7 μm thick. Tissue sections were mounted on the slides. After drying, sections were stained with haematoxylin-eosin stain by following standard protocol and slides were mounted in D.P.X.

Results: The nomenclature of the olfactory system was implemented as used earlier (Hofmann and Meyer, 1991; Meyer et al., 1996, Lazar et al., 2004). The olfactory organs are covered with dorsal portion of the rostral chondrocranium. At the rostral end of the olfactory organ, the epithelium is divided into left and right. The longitudinal sections through olfactory system of adult frog *M. ornata* showed the main olfactory chamber and vomeronasal organ. The main olfactory chamber is further divided in to medial diverticulum and lateral diverticulum (Fig. 1, A-D). Medial olfactory chamber is found to be cylindrical (Fig. 1 A-C) and olfactory nerves originated from olfactory epithelium and terminated in to main olfactory bulb (Fig. 1 A). The main olfactory bulb was divided in to three layers, glomerular layer, mitral cell layer and internal granular layer (Fig. 1 A) and it receives nerve fibers from olfactory epithelium of main olfactory system. Epithelium of medial diverticulum of consists of Bowman's gland and three kinds of cells i.e. basal cells, olfactory receptor neurons and supporting cells (cellular details not seen). Lateral diverticulum was situated in the lateral side of the medial diverticulum (Fig. 1 E). Vomeronasal organ is situated at rostral end of olfactory system. Vomeronasal organ was seen to be bean shape and consisting of vomeronasal cavity and vomeronasal epithelium (Fig. 1 B and D). Vomeronasal organ is surrounded by the round cells called as vomeronasal glands. Vomeronasal nerve originated from the vomeronasal organ and innervated to accessory olfactory bulb. In transverse sections, main olfactory bulb situated in anterior end of telencephalon and accessory olfactory bulb was observed on ventrolateral side of main olfactory bulb (Fig. 1 F). The left and right part of olfactory system is separated by cartilage nasal septum and medial diverticulum olfactory system was cylindrical in shape (Fig. 1 G, I) and magnified view main olfactory system showing olfactory epithelium (Fig. 1 H).

Figure 1

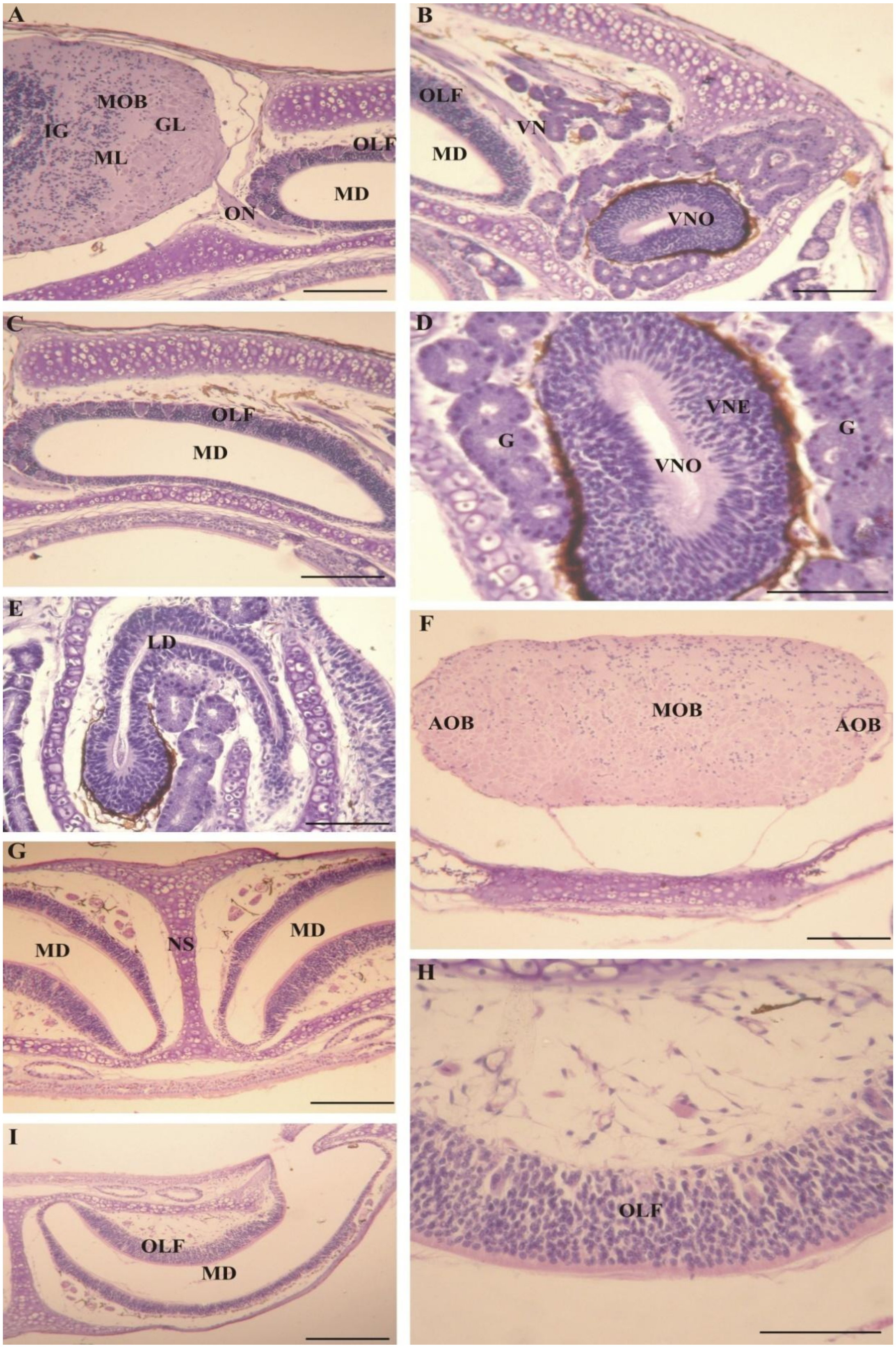

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Figure 1. The longitudinal and transverse sections through of olfactory system of adult frog *M. ornata* showed the organization of main olfactory system and vomeronasal system. A: The main olfactory bulb consists of three layers, glomerular layer, mitral cell layer and internal granular layer. C: Medial olfactory chamber is seen to be cylindrical. E: Lateral diverticulum. B and D: Vomeronasal organ is consisting of vomeronasal cavity, vomeronasal epithelium and surrounded by vomeronasal glands (G). F: Transverse section of olfactory bulb shows main olfactory bulb and laterally located accessory olfactory bulb. G and I, The left and right part of olfactory system is separated by cartilage nasal septum and medial diverticulum olfactory system is found to be cylindrical. H: The magnified view showed olfactory epithelium. Scale bar, A-C, E-G, I, 15 μm, D, H, 25μm.

Discussion: All the organisms are exposed to a continuous flow of olfactory sensory information. Depending on the chemical signal detected, olfactory information can be processed by two different systems, the main olfactory system and accessory olfactory system (Moulton and Beidler, 1967). The two divisions of the olfactory system appear to be used alternately by the frog. The middle cavity/lateral diverticulum, is always filled with water and serves the animal when it is in water (Altner, 1962) while the principal cavity/medial diverticulum is filled with air and appears to function when the animal is in terrestrial surroundings (Weiss, 1986). The olfactory system of frog *M. ornata* consists of main olfactory system and vomeronasal organ. The main olfactory system is divided in to the medial and lateral diverticulum. The similar morphological organization of the olfactory system was reported in *X. laevis* on the basis of lactin binding properties (Hofmann and Meyer, 1991). The medial diverticulum is found to be a long cylindrical in *M ornata* as reported in other anurans (Scalia, 1976; Holtzman and Halpern, 1990; Eisthen et al., 1994; Reiss and Burd, 1997). The sensory receptors in the lateral diverticulum function as chemosensory detectors in aquatic environments in *X. laevis* (Reiss and Burd, 1997) whereas lateral diverticulum is non sensory in typical frogs. Weather the lateral diverticulum in frog *M ornata* is either sensory or nonsensory is not known. Further study is required to elucidate functional organization of olfactory system in *M. ornata*. The sensory neurons were situated in epithelium inside the nasal cavity and the axons projects to glomerular structures in the olfactory bulb at the rostral end of the telencephalon in *M. ornata.* Similar organization was reported in other amphibians (Schmidt and Wake, 1990). In frog *M. ornata* olfactory bulb was seen to be divided in to three cell layers glomerular layer, mitral cell layer and internal granular layer. Similar structural organization of olfactory bulb was reported in *Rana esculenta* (Lazar et. al., 2004) and in the other frogs, which receives the olfactory nerve. It is reported that olfactory epithelium express a different odorant receptors and send their axons to glomeruli in distinct regions of olfactory bulb (Reiss and Burd, 1997).

The vomeronasal system is a specialized olfactory system found in almost all tetrapods. It is implicated for detection of nonvolatile stimuli including pheromones and also involved in sexual behavior (Baxi et al., 2006; Reiss and Eisthen, 2007). The vomeronasal organ is absent in some vertebrates while well developed in snakes, lizards and rodents (Brennan, 2001). In the *M. ornata*, vomeronasal organ is situated on ventrolateral side of main olfactory system. Present study has shown that vomeronasal organ is oval/bean shape in *M. ornata*. Similar structural organization was observed in other anurans. Vomeronasal nerve fibers were originated from vomeronasal epithelium and communicating with accessory olfactory bulb situated at caudal end of main olfactory bulb. Similar appearance has been reported in amphibians (Saito et al., 2006). This position and organization of vomeronasal organ indicates that it may be involved in the perception of fluid phase stimuli. Several controversial interpretations have been made on the evolution of vomeronasal organ in amphibians. Bertmar (1981) hypothesized that the vomeronasal organ evolved in tetrapods as an adaptation for terrestrial habitat. Some workers support the monophyly of amphibians and suggest that the most recent common ancestor to the tetrapods was aquatic (Ahlberg and Milner, 1994) and tetrapods have lost the vomeronasal organ during evolution (Eisthen, 2000).

Majority of amphibians spend most of their time in the interface between air and water. In air-water interface the concentration of substances is higher than in air alone or in water. Therefore, this interface is a favorable environment for distribution of chemical signals. Present knowledge on the role of the air/water interface and its importance for chemocommunication in amphibians is not understood. To smell both water and air borne molecules in amphibians is received by two distinct regions of olfactory epithelium. Furthermore olfactory system is an ancient chemosensory system and organization of this chemosensory system has been phylogenetically conserved.

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