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Morphological study on the olfactory systems of the snapping turtle, *Chelydra serpentina*

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ABSTRACT

In this study, the olfactory system of a semi-aquatic turtle, the snapping turtle, has been morphologically investigated by electron microscopy, immunohistochemistry, and lectin histochemistry. The nasal cavity of snapping turtle was divided into the upper and lower chambers, lined by the sensory epithelium containing ciliated and non-ciliated olfactory receptor neurons, respectively. Each neuron expressed both Gαolf, the α-subunit of G-proteins coupling to the odorant receptors, and Gαo, the α-subunit of G-proteins coupling to the type 2 vomeronasal receptors. The axons originating from the upper chamber epithelium projected to the ventral part of the olfactory bulb, while those from the lower chamber epithelium to the dorsal part of the olfactory bulb. Despite the identical expression of G-protein α-subunits in the olfactory receptor neurons, these two projections were clearly distinguished from each other by the differential expression of glycoconjugates. In conclusion, these data indicate the presence of two types of olfactory systems in the snapping turtle. Topographic arrangement of the upper and lower chambers and lack of the associated glands in the lower chamber epithelium suggest their possible involvement in the detection of odorants: upper chamber epithelium in the air and the lower chamber epithelium in the water.

1. Introduction

In general, tetrapods have two olfactory systems: the main olfactory system and the vomeronasal system (Taniguchi and Taniguchi, 2014; Ubeda-Bañon et al., 2011). The olfactory epithelium, a peripheral receptor organ of the main olfactory system, projects axons to the main olfactory bulb. The vomeronasal organ, a peripheral receptor organ of the vomeronasal system, projects axons to the accessory olfactory bulb. The olfactory epithelium and the sensory epithelium of the vomeronasal organ include olfactory receptor neurons, the olfactory cells and the vomeronasal receptor cells, distinguished by the ultrastructure and the expression of olfactory receptors. The olfactory cells have cilia at the tip of the dendrite and express odorant receptors, while the vomeronasal receptor cells have microvilli at the tip of the dendrite and express vomeronasal receptors. The odorant receptors and the vomeronasal receptors are the members of G-protein-coupled receptors; the odorant receptors bind to Gαolf, and the type 1 and type 2 vomeronasal receptors to Gαi-2 and Gαo, respectively (Buck and Axel, 1991; Dulac and Axel, 1995; Herrada and Dulac, 1997).

We have been studying the olfactory systems of several vertebrates in order to elucidate the phylogenetic development of the olfactory systems (Endo et al., 2011; Ibrahim et al., 2013, 2014, 2015; Kondoh et al., 2010, 2011, 2013; Nakamuta et al., 2010a, 2013a,b, 2011, 2012). Unlike the case of mammals, relatively small numbers of studies have been published with regard to the olfactory system of fish, amphibians, reptiles, and birds. Therefore, their morphological details are not fully understood at present. Especially, for the fine structure of the olfactory systems of turtles, only a few among 300 turtle species have been reported to date (gopher tortoise and common box turtle: Graziadei and Tucker, 1970; Reeve's turtle: Hatanaka, 1992; Hatanaka et al., 1982; Matsuzaki, 1982; Taniguchi et al., 1996; Wakabayashi and Ichikawa, 2008; soft-shelled turtle: Nakamuta et al., 2016). In this study, among the turtles present in Japan, we examined the olfactory system of the snapping turtle *Chelydra serpentina*. Snapping turtles, belong to the family Chelydridae, are found in North America and becoming a problem as an invasive species in Japan (Kato and Etoh, 2012; Kobayashi et al., 2006; Steyermark et al., 2008).

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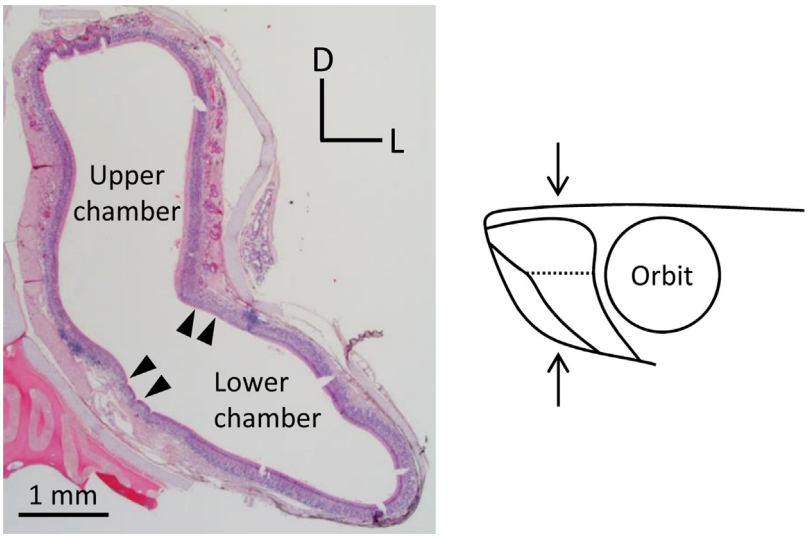

Fig. 1. Transverse section of the nasal cavity showing the upper and the lower chambers lined by the sensory epithelium and separated by the non-sensory epithelium (arrowheads). Right side of the figure is lateral (L), and the upper, dorsal (D). Illustration shows a lateral view of the olfactory organ. Arrows indicate the level of sectioning.

Table 1
Details of animals used in this study.

	Sex	Body weight (g)	Carapace length (cm)
a	male	8300	31.68
	female	1520	17.94
	female	1910	19.88
b	male	705	14.76
	female	690	14.32
c	male	444.7	13.04
	female	1370	17.78
	not determined	14.9	3.6

a: Fixed in 2.5% glutaraldehyde for transmission and scanning electron microscopy; b: fixed in 4% paraformaldehyde for immunohistochemistry; c: fixed in Bouin's solution without acetic acid for lectin histochemistry.

The aim of the present study is to demonstrate the tissue organization of the olfactory system in snapping turtles and to clarify the fine structure of the olfactory receptor neurons by light and electron microscopy. In addition, we aimed to investigate the possible presence of subsystems with distinct properties in the olfactory system of snapping turtle by demonstrating the localization of G-proteins by dual immunofluorescence microscopy and the expression of glycoconjugates by lectin histochemistry.

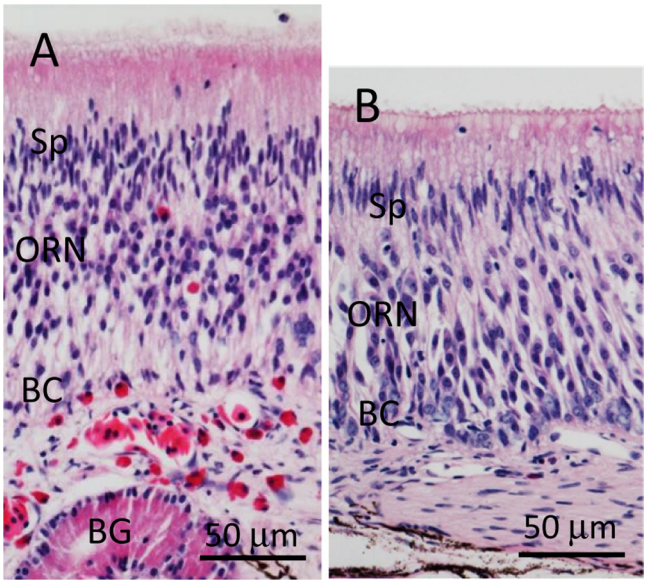

Fig. 2. Higher magnification views of the upper chamber epithelium (A) and the lower chamber epithelium (L), showing nuclei of the supporting cells (Sp), olfactory receptor neurons (ORN), and basal cells (BC). BG indicates the Bowman's glands.

2. Materials and methods

The olfactory organs and olfactory bulbs of the juvenile and adult snapping turtles captured in Shizuoka prefecture, Japan, during May through June 2015, were used (Table 1). Animals were anesthetized by intraperitoneal injection of sodium pentobarbital (64.8 mg/kg). Samples were collected from animals sacrificed by decapitation and processed for transmission and scanning electron microscopy as described before (Nakamuta et al., 2016). Other samples were collected from animals killed by the cardiac perfusion of fixatives and processed for immunohistochemistry or lectin histochemistry as described before (Nakamuta et al., 2016, 2013a). All animals were treated in accordance with the Guide for the Care and Use of Experimental Animals at Iwate University and Shizuoka University.

For the immunohistochemical analysis, a mouse monoclonal anti-Gαolf antibody (sc-55545, Santa Cruz, Dallas, TX, U.S.A.) and a rabbit polyclonal anti-Gαo antibody (551, MBL, Nagoya, Japan) were used. Fluorescent-labeled secondary antibodies used to detect them were Alexa 488-labeled donkey anti-rabbit antibody and TRITC-labeled donkey anti-mouse antibody. Control staining was performed by the use of phosphate-buffered saline (PBS) to replace the primary antibodies. No specific staining was observed in the control slides (data not shown).

For lectin histochemistry, *Dolichos biflorius* agglutinin (DBA) and *Erythrina cristagalli* lectin (ECL) in the biotinylated lectin kits (Vector Laboratories, Burlingame, CA, U.S.A.) were used. Control staining was performed by the use of PBS to replace the biotinylated lectins. No specific staining was observed in the control slides (data not shown).

3. Results

The nasal cavity was divided into the upper and lower chambers separated from each other by medial and lateral ridges covered