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Olfactory Receptor Neurons in Fish: Structural, Molecular and Functional Correlates

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Introduction

In rodents, the olfactory system is segregated into the main olfactory epithelium (OE) and into the vomeronasal organ (VNO), the former populated by ciliated olfactory receptor neurons (ORNs) expressing the G-protein subunit $G\alpha_{olf}$ and OR-type odorant receptor molecules, the latter housing microvillous receptor neurons (VRNs) expressing $G\alpha_o$ and $G\alpha_i$ and V1R, V2R and V3R receptor molecules. Projections of ORNs and VRNs target distinct areas in the olfactory bulb (OB) and the accessory olfactory bulb (AOB), respectively.

Differing from the situation in rodents, the fish OE contains three different types of ORNs: ciliated, microvillous and crypt cells. These three types of ORNs are intermingled in one epithelium. Recently, Nikonov and Caprio (2001) showed that in catfish responses to biologically relevant stimuli form a consistent map in the OB. The present study asked whether a distinct function can be attributed to the different types of ORNs; whether the different types of ORNs utilize different G-protein α-subunits and different OR or V2R receptor molecules; and whether the different morphological types of ORNs are distributed homogeneously across the olfactory lamellae.

Materials and methods

The study was carried out in the catfish (*Ictalurus punctatus*) and the goldfish (*Carassius auratus*). We injected the fluorescent tracer DiI into OBs to retrogradely label ORNs in the OE. Immunocytochemistry was used to examine the expression of G-protein subunits. Probes for OR-type and V2R-type receptors [courtesy of John Ngai, UC Berkeley (catfish OR probes), Michelle Rankin, NIH (catfish V2R probe) and Y. Cao and L. Stryer, UCSF (goldfish OR and V2R probes)] were used for *in situ* hybridization to visualize the expression of odorant receptor molecules. All three methods were carried out at the light microscopic and the electron microscopic level. All procedures were carried out with the approval of the Institutional Animal Care and Use Committees of the respective institutions.

Results and discussion

Our results as shown by DiI injections indicate that ciliated ORNs predominantly project to medial and ventral areas of the OB, areas for which Nikonov and Caprio (2001) reported responses to bile salts. DiI injections into the posterior dorsal areas labeled microvillous ORNs in the OE. In the posterior dorsal areas of catfish Nikonov and Caprio (2001) found responses to nucleotides. DiI injections into anterior dorsal areas retrogradely labeled microvillous ORNs while injections into anterior ventral areas labeled ciliated ORNs. For both regions Nikonov and Caprio (2001) reported responses to amino acids. Crypt ORNs were labeled only by injections into two very small distinct ventral areas. Interestingly, catfish and goldfish revealed differences in the expression of G-protein subunits. $G\alpha_{olf}$ was expressed in ciliated ORNs in both species, while $G\alpha_o$ was expressed in crypt ORNs in catfish and in microvillous ORNs in goldfish. Conversely, $G\alpha_q$ was expressed in microvillous ORNs in catfish and $G\alpha_{q/11}$ in crypt cells of goldfish. In addition, goldfish crypt ORNs expressed $G\alpha_o$ in their insunk cilia—the only case where two different protein subunits were expressed in one cell type (Hansen *et al.*, 2003, 2004).

In both catfish and goldfish OR-type probes labeled ciliated ORNs while V2R-type probes labeled microvillous ORNs. Neither set of probes available labeled crypt ORNs. Based on the results of G-protein subunit immunoreactivity and *in situ* hybridization in whole mount preparations of lamellae, the distribution of ciliated ORNs is more or less homogeneous across the lamellae of goldfish but heterogeneous in catfish. The distribution of microvillous and crypt type ORNs is heterogeneous in both catfish and goldfish. ORNs in both catfish and goldfish never expressed more than one molecular receptor per cell. In goldfish, V2R probes were co-expressed with $G\alpha_o$ (Hansen *et al.*, 2003, 2004). In catfish, bile salts are detected by ciliated ORNs, nucleotides by microvillous ORNs and amino acids by ciliated and microvillous ORNs. A recent study by Sato and Suzuki (2001) reported a similar scenario for trout. The function of crypt ORNs is unknown. We conclude that in catfish, distinct types of ORNs project to defined areas in the olfactory bulb. G-protein α-subunits mediate the transduction pathways in catfish and goldfish, but different subunits are expressed in different cell types. In both species OR-type receptor molecules are expressed in ciliated ORNs, and V2R-like receptor molecules are expressed in microvillous ORNs.

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