Chem. Senses 30 (suppl 1): i58–i59, 2005

doi:10.1093/chemse/bjh112

Development of the Vagal Taste System of Goldfish

Charles Lamb[1] and Sadao Kiyohara[2]

[1]Department of Science, Black Hills State University, Spearfish, SD 57783, USA and [2]Department of Chemistry and Bioscience, Faculty of Science, Kagoshima University, Kagoshima 890-0065, Japan

Correspondence to be sent to: Dr Charles Lamb, e-mail: CharlesLamb@bhsu.edu

Key words: development of taste, lamination, taste bud, topography, vagal lobe

Introduction

Goldfish are among a group of fishes which possess a highly developed sense of taste. Many species of the cyprinid (minnow) and catostomid (sucker) families possess specialized food-handling structures in the oropharynx (e.g. the palatal organ and branchial arches) which are covered with taste buds innervated by fibers of the glossopharyngeal and vagal nerves. The central termination for these gustatory afferents is an enlarged lobe on the dorsal surface of the hindbrain (vagal lobe). The vagal lobe is a highly organized, laminated structure consisting of an outer sensory zone, a central fiber zone, and an inner zone of vagal motor neurons (Morita *et al.*, 1983). Gustatory afferents from the oropharyngeal epithelium travel into the vagal lobe through the fiber zone to terminate topographically in restricted portions of the sensory zone (Morita and Finger, 1985). Interneurons in the sensory layers project radially inward to the motor zone, connecting sensory input from a given portion of the sensory epithelium with motor neurons projecting to muscles in that same region of the oropharynx (Finger, 1988). This results in a highly ordered neural system organized in radial 'modules' that are topographically related to the sensory epithelium.

While general development of the goldfish has been studied previously, and taste bud development in zebrafish (Hansen *et al.*, 2002), little is known of the development of the central connections of the vagal nerve and of the vagal lobe in cyprinids. Our lab is investigating the development of the goldfish vagal taste system, and the relationships of peripheral and central developmental mechanisms.

Materials and methods

Goldfish were raised from fertilized eggs to 6 months of age (~30 mm total length) in the laboratory (21°C). Live fish were monitored daily and analyzed for gross development and for behavioral development throughout the study. Specimens were collected every 6 h through hatching, then daily to 1 month and weekly thereafter. They were briefly anaesthitized in MS-222 and fixed in 4% paraformaldehyde (in 0.1 M PO_4 buffer) for subsequent analyses. Whole-mount specimens or removed brains were embedded in paraffin and cut transversely at 5–10 µm. Immunohistochemistry was used to identify nerve processes (β-tubulin, HNK-1) or proliferating cells (PCNA, BrdU). Additional specimens were labeled with DiI, a neural tracer, injected into the taste epithelium or the vagal ganglion. These specimens were incubated at 37°C for 1–5 days then sectioned at 50 µm on a Vibratome and viewed under epifluorescence. To further study cell proliferation in the vagal lobe, we exposed fish of different ages (6–78 days) to $[^3H]$thymidine and processed them at intervals varying from 2 months to 6 months following exposure.

Results

Goldfish hatch at ~4 days post-fertilization (4 mm). By 1 week of age (6 mm), larvae have absorbed most of the yolk sac and are actively feeding in the water column, the branchial region is developing as the mouth moves to a more terminal position, otoliths are present, and the swim bladder is enlarging. Taste buds, which are first evident in the oropharynx by 6 days, start to show elongated cells. The palatal organ forms, as both the muscle layer and the epithelium thicken. Motor cells begin to differentiate at the base of the presumptive vagal lobe and processes can be seen entering the vagal root.

Goldfish at 3 weeks of age (8 mm) are considered juveniles, as the body structure and swimming patterns take on adult characteristics. Taste buds are numerous throughout the oropharynx, and most are morphologically mature. A vagal motor root is present, as more motor neurons are differentiating, and a region of neuropil develops between the motor zone and undifferentiated cells on the lateral surface of the lobe. Cell proliferation is most prominent in the ventricular region of the dorsal medulla and along the midline.

By 4–5 weeks (9 mm), the palatal organ is adult-like, with a large muscle mass and fully mature taste buds. Cells on the medial edge of the superficial cell layer of the vagal lobe send processes radially inward to the neuropil layer. Distinct sensory and motor fibers are evident and motor neurons are migrating ventrolaterally toward the root.

By 2–3 months (15 mm), lamination is first evident as layers of cells on the dorsolateral surface of the lobe start to migrate medially into the neuropil. There are three distinct fiber groups present: medial motor fibers, a central sensory root with fibers which branch off laterally to terminate in the newly forming sensory layers, and a superficial sensory root which travels along the border of the neuropil and the undifferentiated cells on the dorsolateral surface of the lobe. Proliferation occurs mainly at the tip of the vagal lobe, but also along the midline and on the lateral surface. This pattern continues through adulthood, as cells are continually being added to the vagal lobe at these locations. Cells produced at the tip of the lobe form radial cohorts that are oldest at the base of the lobe and are progressively younger more dorsally.

At 5–6 months (25 mm), the vagal lobe is approaching the adult morphology. The sensory zone is much larger as cells continue to migrate medially from the superficial cell layers, and there is a distinct fiber zone separating the sensory and motor layers. As in the adult, most of the vagal afferents now enter the central sensory root and the superficial root becomes less pronounced.

Conclusions

In goldfish, the vagal lobe develops very late relative to the rest of the oropharyngeal taste system. By 3 weeks after fertilization (7 mm), taste buds are innervated and are maturing, as is the palatal organ musculature. Larvae at this stage also display adult-like feeding behaviors including intake and rejection of particles in the water column. The dorsal medulla consists of a region of undifferentiated cells overlying the central processes of the vagal and glossopharyngeal afferents. Increased proliferation on the dorsal surface of the medulla forms the anlage of the vagal lobe by 3 weeks, with a subsequent increase in neuropil which will become the deeper sensory layers of the lobe. Growth of the vagal lobe occurs dorsally as new cells are added to the dorsal tip. Cells proliferate in radial layers, with motor neurons and interneurons at a particular region of the lobe differentiating simultaneously. This mechanism of proliferation of radial cohorts of cells to form a laminar neural structure is different from the mechanisms present in mammals, but is similar to the development of the optic tectum in fish (Nguyen *et al.*, 1999).

Cells start migrating into the neuropil of the vagal lobe at 4 weeks (9 mm), with immature lamination appearing in the sensory zone by 2–3 months (15 mm). It is not clear what the relationship is between differentiation and lamination, but there is a significant delay. The sensory root consists of two groups of fibers: a superficial root and a fasciculated central root.

The vagal lobe matures morphologically at 5–6 months (25 mm), as the sensory zone increases in thickness with the addition of cell layers and the central sensory root becomes the predominant source of afferent input. Future work should investigate how the functional development of the vagal taste system relates to the morphological development, and how this process is molecularly controlled.

Acknowledgements

The authors thank Dr Tom Finger, in whose lab much of the work was done and whose support was substantial (NIH Grant DC00147). This study has been supported by Black Hills State University Faculty Research Awards (C.L.) and by grant-in-aid 16380137 from the Ministry of Education, Culture, Sports, Science, and Technology—Japan (S.K.).

References

Finger, T.E. (1988) *Sensorimotor mapping and oropharyngeal reflexes in goldfish,* Carassius auratus. Brain Behav. Evol., 31, 17–24.

Hansen, A., Reutter, K. and **Zeiske, E.** (2002) *Taste bud development in the zebrafish,* Danio rerio. Dev. Dyn., 223, 483–496.

Morita, Y. and **Finger, T.E.** (1985) *Topographic representation and laminar organization of the vagal gustatory system in the goldfish,* Carassius auratus. J. Comp. Neurol., 264, 231–249.

Morita, Y., Ito, H. and **Masai, H.** (1983) *Cytoarchitecture and topographic projections of the gustatory centers in a teleost,* Carassius carassius. J. Comp. Neurol., 218, 378–391.

Nguyen, V., Deschet, K., Henrich, T., Godet, E., Joly, J.-S., Wittbrodt, J., Chourrout, D. and **Bourrat, F.** (1999) *Morphogenesis of the optic tectum in the medaka (*Oryzias latipes*): a morphological and molecular study, with special emphasis on cell proliferation*. J. Comp. Neurol., 413, 385–404.