

Taste System in the Axolotl: Physiology and Anatomy

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For single cell recordings and various experimental manipulations, physiologists prefer an animal model having large somatic cells and a competence to survive harsh surgery. Salamanders fit these requirements. Among various species of salamanders, the mudpuppy (*Necturus*), the tiger salamander (*Ambystoma tigrinum*), and the axolotl (*Ambystoma mexicanum*) have often been used in physiological experiments. The use of the axolotl for neurobiology has been reviewed by Harris in a recent volume (Armstrong and Malacinski 1989). Salamanders are also useful for studying neural mechanisms of "taste." This article may be regarded as another chapter on the neurobiology of taste in salamanders based on our recent findings in this line obtained from the axolotl in particular.

Mudpuppy. Much of our knowledge of the taste system in the salamander comes from the mudpuppy. Using a glass microelectrode, West and Bernard (1978) impaled single taste cells in the lingual epithelium of the mudpuppy, and studied the electrophysiological properties of the cell membrane in response to stimulation by various chemicals. Possibly due to the large size of the cell, the loss of excitability was minimal, and thus it was possible to demonstrate a regenerative spike in the taste cell (Roper, 1983). Effective stimulants were surveyed by extracellular recordings from the glossopharyngeal (IX) nerve (Samanen and Bernard, 1981), which together with the facial (VII) nerve and the vagus (X) nerve constitute the three pairs of cranial gustatory nerves. HCl (sour), NaCl (salty), and quinine hydrochloride (bitter) were effective. Sucrose (sweet) was least effective. Staining of single taste cells with fluorescent dye successfully showed a functional coupling between these cells (West and Bernard, 1978; Yang and Roper, 1987). An excitatory property of the taste cell membrane can be studied in greater detail if a whole-cell clamping (a new technique in electrophysiology) is applied. To this end, single taste cells

were isolated enzymatically from the mudpuppy (Kinnamon et al. 1988), and also from the tiger salamander (Sugimoto and Teeter 1987) to record chemically induced membrane currents.

A taste bud is a cuboidal structure of collected taste cells. In mammals, the taste buds are embedded in a folded lingual epithelium. Thus the apical side of taste cells is mostly invisible without dissection. In the mudpuppy, however, they are embedded in a flat lingual epithelium and face directly onto the oral cavity. Such a configuration of the taste buds permits easier access for the microelectrodes as well as for the chemicals for gustatory stimulation or fixation. The electron microscope revealed the fine structure of the taste buds in the apical microvilli (Cummings et al. 1987) and three types of taste receptor cells based on intracellular structures and synapses (Farbman and Yonkers 1971; Delay and Roper 1988). There are a few neuroanatomical studies on the central projection of gustatory nerves in salamanders (Herrick 1948; Opdam and Nieuwenhuys 1976; Roth and Wake 1985), but no electrophysiological study on higher gustatory neurons in the salamander brainstem.

Axolotl. We have adopted the axolotl as a model animal to study the physiology and anatomy of the taste system (Nagai et al. 1989), because of the availability of inbred strains, the relative ease with which it can be raised in a laboratory, and the cooperation of the Indiana University Axolotl Colony (practically speaking most important). Juveniles of the axolotl have been regularly shipped by air cargo to our laboratory in Tokyo. We raise them at normal room temperature for several months to a year by feeding them dog food pellets. Thus in our aquarium, axolotls at various developmental stages are ready for use year round. They stay healthy even in summer. These are the advantages of using axolotls, compared to mudpuppies or tiger salamanders, which must be captured in the field, and which require special temperature controls to maintain them in a healthy condition. We usually use the adult in surgical procedures for physiological recordings and neuroanatomy.

We recorded the neural responses from the glossopharyngeal (IX) nerve of axolotls (Nagai, 1989). The integrated whole nerve recordings provided a response profile to salts (0.5 M KCl, 1.0 M NaCl, 0.5 M CaCl₂), bitter (1

Axolotl Newsletter Number 19

mM quinine-HCl), acid (0.1 N citric acid), sweet (1.0 M sucrose) and amino acids (50 mM, Phe, Arg, Gly, Ala, Glu). Generally the response to CaCl_2 was robust, but not that to amino acids and sucrose. The response to quinine and citric acid was smaller than that to 0.5 M KCl. We were interested in knowing if the taste response differs among four inbred strains (wild, white, albino, and eyeless). The result turned out to be rather discouraging. The response profiles to the chemicals listed above seemed similar in the four strains. We need to pursue the study of chemosensitivity among the strains using more animals and chemicals, however, because the studies on chemosensitivity in the mudpuppy are contradictory (Samanen and Bernard 1981; McPheeters and Roper 1985), and there may be seasonal variation in the properties of the taste cell membrane (Roper and McBride 1989). Because the axolotl is a carnivore, it was suspected that its taste system would be sensitive to amino acids or to a diet containing these. The extracts from dog food and earthworm, however, induced a far smaller response than 0.5 M KCl. The small responses to the amino-acid-containing diets were possibly due to the salts they contained. A question arose: Is the axolotl endowed with discriminative ability with respect to taste qualities? As a test we presented to an axolotl a dog food pellet soaked in quinine solution (above 10 mM). After taking the pellet into the oral cavity, the animal spit it out. This simple behavioral experiment showed that the axolotl could discriminate the bitter substance, which induced a rejection behavior in most animals. The concentration used was high, but we should remember that the experiment was done in the water. The axolotl may be useful in a behavioral study on food intake, because in a separate experiment we found that snapping at prey could be induced if the prey were presented while the water was vibrated at low frequency (2 - 5 Hz). The lateral line nerve of the axolotl detects the vibration (Munz et al. 1984).

The IXth nerve responded not only to chemicals but also to mechanical stimulation. Squirting a water jet onto a single taste bud induced the brisk response of a few units in the whole nerve recording. The taste bud is supposed to be a chemosensory organ, but may also function as a mechanotransducer. In support of this view, basal cells in mudpuppy taste buds resemble Merkel cells (cutaneous mechanoreceptors) (Delay and Roper 1988). Similar basal cells have also been found in

axolotl taste buds (Toyoshima et al. 1987). The mechanosensitive units were larger in amplitude than the chemosensitive ones, suggesting fiber groups with a different diameter and function were contained in the IXth nerve. To identify these neurons (fiber groups) physiologically and then reveal their function in food ingestion behavior in the axolotl, we needed to start with neuroanatomy, i.e., the morphology of the ganglion, where the cell bodies of neurons are distributed, and where their central projection to the brainstem is located. We examined the IXth nerve by a neuronal tracer of cobaltic lysine complex (Co-lys, Nagai and Matsushima 1990). The tracer is useful for studying the details of the neuronal structure in the amphibian, in particular (Oka et al. 1987).

Co-lys was applied to the cut end of the IXth nerve, just distal to the IX-X ganglion complex. The dark-brown end product of Co-lys labeled the cell body and process in the ganglion. The afferent cell bodies in the ganglion were middle-sized (ca. 25 μm). The processes, when successfully labeled, were pseudounipolar. In mammals and frogs, the IXth nerve forms two separate ganglia in the IX-X ganglion complex. In axolotls, however, the ganglion was not clearly divided into two, but formed a combined ganglion. The labeled cells were diffusely distributed in the rostral part of the IX-X ganglion complex. A few labeled cells were also seen in the caudal part, where the vagus (X) nerve fiber and cell bodies were mainly distributed. To reveal the distribution patterns of the IXth nerve and the Xth nerve cell bodies, we applied HRP and Co-lys simultaneously to the respective nerves. The cell bodies of the IXth nerve, discerned by the blue-black end product of HRP, appeared among the numerous Xth nerve cell bodies in the caudal part. Hence, in the axolotl, the ganglion cells of the IXth nerve are not clearly separated from those of the Xth nerve.

The IXth nerve system in the axolotl assumes a primordial form in its ganglion, but its topographical organization in the brainstem is basically similar to that in frogs. The afferent and efferent components of the IXth nerve in the brainstem were transganglionically labeled with Co-lys. The majority of the afferent fibers formed strong ascending and descending bundles in the solitary fasciculus. The rest of the afferent fibers formed in the spinal tract of the trigeminal nerve a weak bundle, which had a short ascending limb and a long descending limb. These two bundles had terminal areas in

the ipsilateral brainstem: in the dorsal gray matter for the solitary fasciculus and in the lateral funiculus for the spinal tract of the trigeminal nerve, respectively. The cell bodies of the efferent neurons had developed dendritic arborizations in the ventrolateral white matter, and formed a longitudinal cell column in the ventrolateral margin of the gray matter.

Literature Cited

- Armstrong, J. B., and G. M. Malacinski. 1989. *Developmental biology of the axolotl*. Oxford University Press, Oxford.
- Cummings, T. A., R. J. Delay, and S. D. Roper. 1987. Ultrastructure of apical specializations of taste cells in the mudpuppy, *Necturus maculosus*. *J. Comp. Neurol.* 261:604-615.
- Delay, R. J., and S. D. Roper. 1988. Ultrastructure of taste cells and synapses in the mudpuppy, *Necturus maculosus*. *J. Comp. Neurol.* 277:268-280.
- Farbman, A. I., and J. D. Yonkers. 1971. Fine structure of the taste bud in the mudpuppy, *Necturus maculosus*. *Am. J. Anat.* 131:353-370.
- Herrick, C. J. 1948. *The Brain of the Tiger Salamander*. Univ. Chicago Press, Chicago.
- Kinnamon, S. C., T. A. Cummings, and S. D. Roper. 1988. Isolation of single taste cells from lingual epithelium. *Chem. Senses.* 13:355-368.
- McPheeters, M., and S. D. Roper. 1985. Amiloride does not block taste transduction in the mudpuppy, *Necturus maculosus*. *Chem. Senses.* 10:341-352.
- Munz, H., B. Claas, and B. Fritsch. 1984. Electrorceptive and mechanoreceptive units in the lateral line of the axolotl *Ambystoma mexicanum*. *J. Comp. Physiol. A* 154:33-44.
- Nagai, T. 1989. Chemoresponses of the glossopharyngeal nerve of the axolotl (*Ambystoma mexicanum*). *Chem. Senses.* 14:315 (Abstract).
- Nagai, T., T. Matsushima, and K. Toyoshima. 1989. Axolotl: a model system for taste research. *Chem. Senses.* 14:324 (Abstract).
- Nagai, T., and T. Matsushima. 1990. Morphology and distribution of the glossopharyngeal nerve afferent and efferent neurons in the Mexican salamander, Axolotl: a cobaltic lysine study. (Submitted).
- Oka, Y., H. Takeuchi, M. Satou, and K. Ueda. 1987. Cobaltic lysine study of the morphology and distribution of the cranial nerve efferent neurons (motoneurons and preganglionic parasympathetic neurons) and rostral spinal motoneurons in the Japanese toad. *J. Comp. Neurol.* 259:400-423.
- Opdam, P., and R. Nieuwenhuys. 1976. Topological analysis of the brain stem of the axolotl *Ambystoma mexicanum*. *J. Comp. Neurol.* 165:285-306.
- Roper, S. 1983. Regenerative impulses in taste cells. *Science* 220:1311-1312.
- Roper, S. D., and D. W. McBride, Jr. 1989. Distribution of ion channels on taste cells and its relationship to chemosensory transduction. *J. Memb. Biol.* 109:29-39.
- Roth, G., and D. B. Wake. 1985. The structure of the brainstem and cervical spinal cord in lungless salamanders (family plethodontidae) and its relation to feeding. *J. Comp. Neurol.* 241:99-110.
- Samanen, D. W., and R. A. Bernard. 1981. Response properties of the glossopharyngeal taste system of the mudpuppy (*Necturus maculosus*). *J. Comp. Physiol.* 143:143-150.
- Sugimoto, K., and J. H. Teeter. 1987. Voltage-dependent and chemically-modulated ionic currents in isolated taste receptor cells of the tiger salamander. *Proc. Soc. Neurosci.* 13:1404 (Abstract).
- Toyoshima, K., K. Miyamoto, and A. Shimamura. 1987. Fine structure of taste buds in the tongue, palatal mucosa and gill arch of the axolotl, *Ambystoma mexicanum*. *Okajimas Folia Anat. Jpn.* 64:99-110.
- West, C. H. K., and R. A. Bernard. 1978. Intracellular characteristics and responses of taste bud and lingual cells of the mudpuppy. *J. Gen. Physiol.* 72:305-326.
- Yang, J., and S. D. Roper. 1987. Dye-coupling in taste buds in the mudpuppy, *Necturus maculosus*. *J. Neurosci.* 7:3561-3565.