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Fine Structure of Taste Buds in the Barbel of the Catfish, *Ictalurus punctatus*

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Summary. Taste buds occur in the epithelium of the catfish barbel along its entire length. Two major cell types, light and dark cells, occupy the upper two-thirds of the taste bud. A third cell type, the basal cell, lies on the basal lamina and is essentially separated from the light and dark cells by a plexus of unmyelinated nerve fibers. The dark cells have branching processes, both apically and basally whereas the light cells have a single apical process and many basal processes. The apical processes of dark cells contain secretory granules, while the apical processes of light cells contain an abundant agranular endoplasmic reticulum. Light cell nuclei contain bundles of 10 nm filaments, often arranged in the shape of a cup or ring, but nucleoli are rarely seen. It is suggested that this morphology indicates a low degree of RNA synthesis by light cells. The basal cells contain large numbers of vesicles, about 60 nm in diameter, which are sometimes seen in clumps in relation to an adjacent nerve fiber in a configuration resembling a synapse. Curiously, although basal cells present a large surface to the basal lamina, there are no hemidesmosomes. This suggests that the basal cell does not originate from the epidermis.

Key words: Taste buds – Fishes – Electron microscopy – Nucleolus.

Introduction

The catfish, *Ictalurus punctatus,* has four pairs of barbels, a *dorsal* pair, located on the dorsal surface of the head; a mandibular pair, the largest ones, located at the corners of the mouth; and two pairs of ventral barbels, the outer one of which is referred to as *lateral* and the inner as *ventral* (Olmsted, 1920).

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Many taste buds are found in the epidermis along the entire length of all of the barbels but the majority is located on the anterior surface, i.e., the leading edge in the direction of swimming.

Because of its accessibility, the catfish taste bud is a convenient model for the study of trophic neuronal influences on epithelial cells. The present study is intended to establish a baseline of normal ultrastructure as a necessary preliminary to studies of taste bud degeneration and regeneration. Several cytological findings not previously reported in teleost taste buds (Desgranges, 1965–72; Reutter, 1971; Welsch and Storch, 1969; and Crisp et al., 1975) are presented.

Materials and Methods

The ventral and lateral barbels of the catfish *Ictalurus punctatus* (20–30 gms body wt.), were immersed in a mixture of 4% glutaraldehyde and 2% OsO_4 in 0.1 M phosphate buffer at pH 7.3. The mixture was prepared by cooling both solutions to 0° C, and the same temperature was maintained thereafter to avoid precipitation of lower oxides of osmium or metallic osmium. The total fixation period in two changes of the ice cold mixture was two hours. The tissue was rinsed in 0.1 M phosphate buffer at 4° C, dehydrated in ethanol, passed through several changes of propylene oxide, and embedded in a mixture of Araldite 502 and Epon 812. Thin sections were placed on Formvar coated grids, double stained with uranyl acetate and lead citrate and examined in a Siemens Elmiskop I electron microscope.

Results

The taste buds on the barbels of *Ictalurus punctatus* are pear-shaped organs measuring 50–80 μm in length and slightly less in width. They are composed primarily of many elongated cells which extend from a depression on the epithelial surface, 20–30 μm in diameter, to approximately 3/4 of the distance toward the basement membrane. Lying on the basement membrane are several basal cells. Between the basal and elongated cells is an essentially cell free zone filled with unmyelinated nerve processes.

Ultrastructurally each taste bud of *Ictalurus punctatus* contains three to five basal cells and a number of cells traditionally classified as "light" or "dark". These terms are based upon light microscopic descriptions of taste buds after formaldehyde fixation. With the method of fixation used in the present study the cytoplasmic density appears similar in all taste bud cells. Two distinct cell types can, nevertheless, still be characterized by certain ultrastructural features.

At their apex, dark cells have short thick microvilli with an electron dense coating, while light cells show a single club-shaped process (Fig. 1). In transverse sections just below the apical surface, the light cells have a round smooth outline whereas the dark cells display irregular, branching, processes interposed between light cells (Fig. 3). In some sections light cells are found directly adjacent to each other.

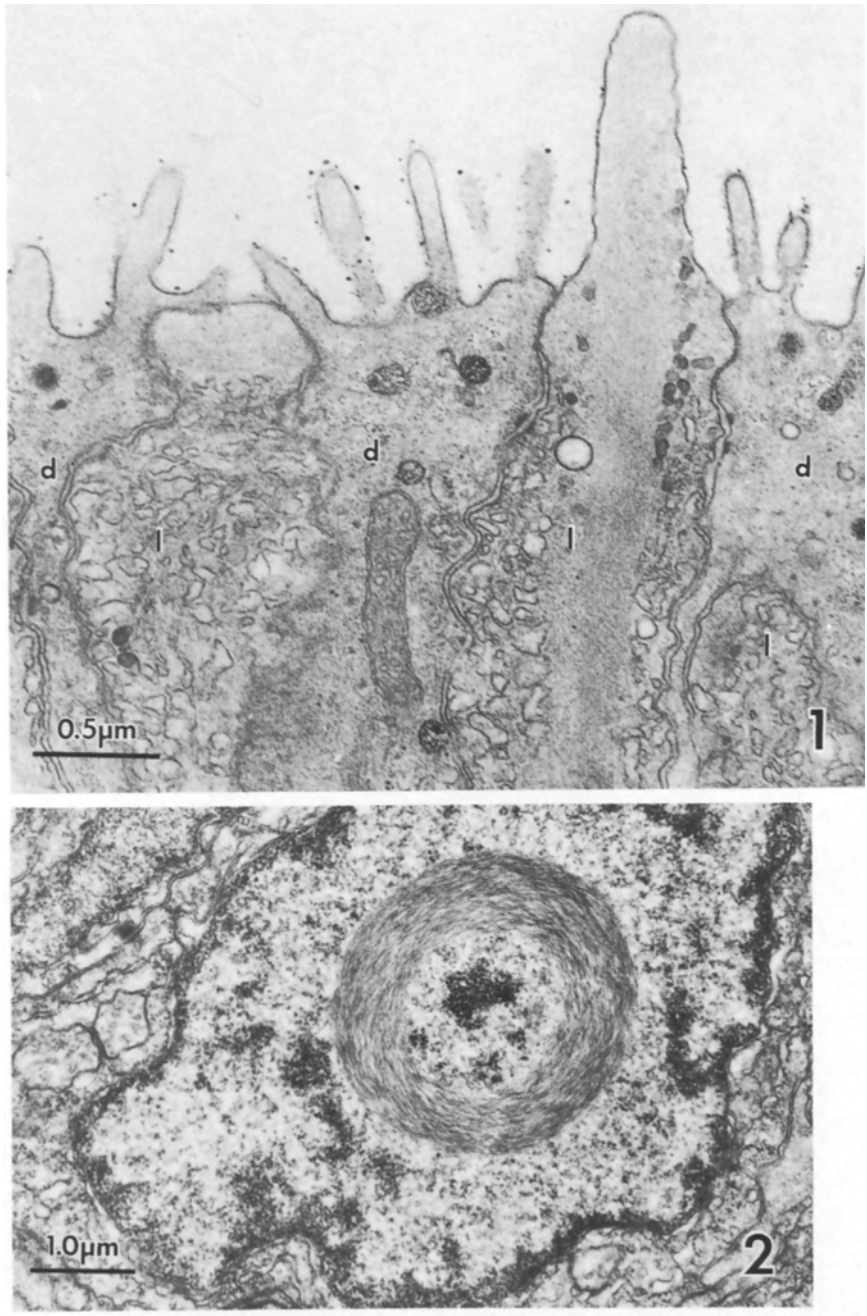
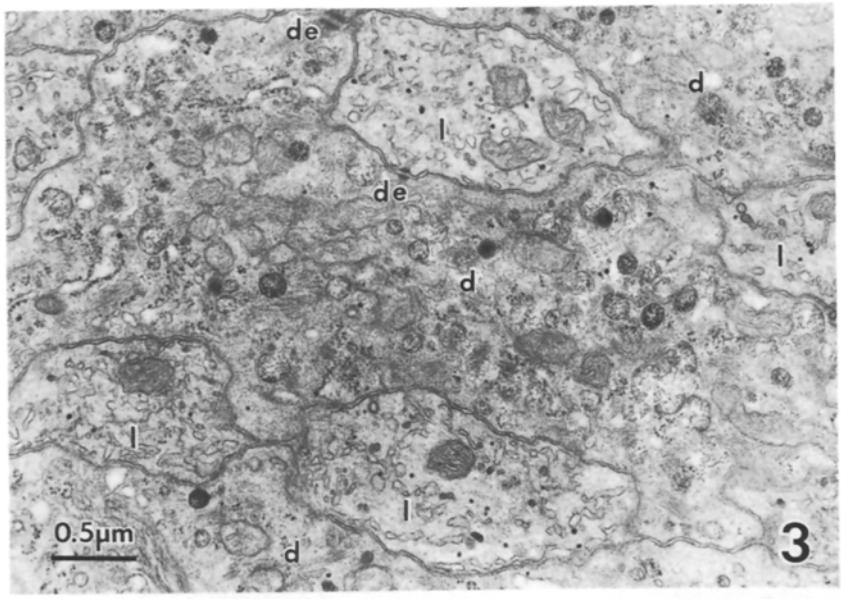


Fig. 1. Detail of apical region of taste bud. Note dark cells (*d*) with several microvilli, light cells (*l*) terminating in a single process. × 34,000

Fig. 2. Light cell nucleus containing ring of filamentous material. × 16,000



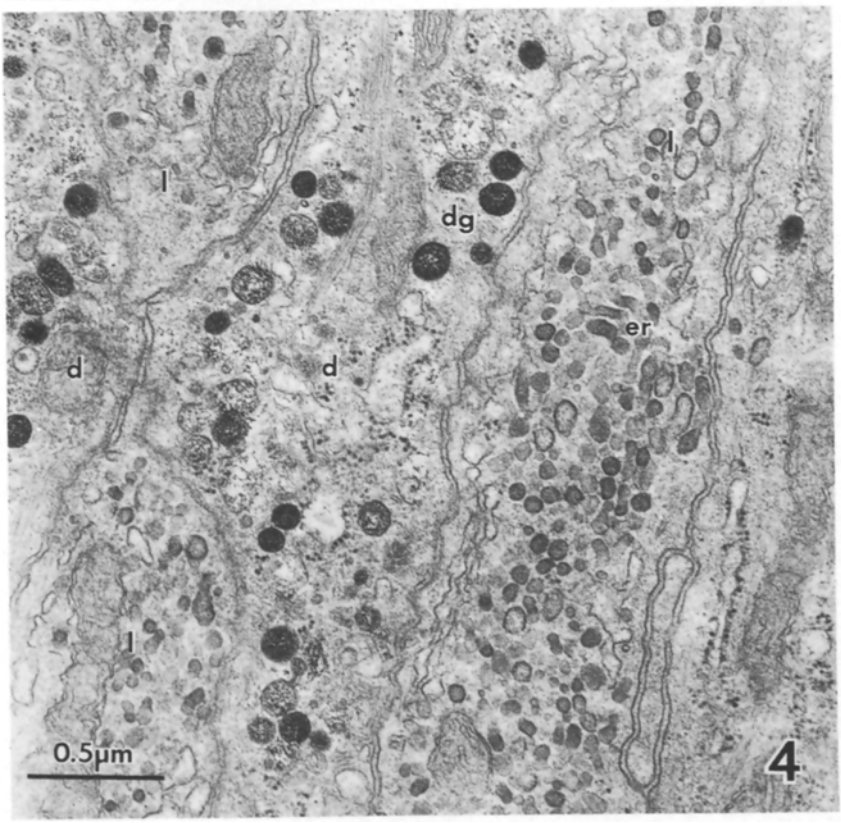


Fig. 3. Transverse section from apical third of taste bud. Note branched apical processes of dark cells (*d*) enclosing light cell (*l*) processes. *de* desmosome. ×22,000

Fig. 4. Longitudinal section through apical processes of dark cells (*d*) containing dense granules (*dg*) and light cells (*l*) containing agranular endoplasmic reticulum (*er*). ×35,000

Montages of electron micrographs of longitudinal sections through the taste buds show that the nuclei of dark cells lie in the middle third of the bud and those of light cells at a deeper level; an observation similar to that in amphibian taste buds (Farbman and Yonkers, 1971; Stensaas, 1971). There are between 60 and 110 light cells in each taste bud. It is difficult to count dark cells dependably because of their irregular profiles. It is estimated, however, from counts on micrographs of sections taken through the nuclear region, that the number of dark cells is approximately equal to that of the light cells.

The dark cells are long and narrow, measuring 50–80 μm from the basal lamina to the apical surface of the taste bud and about 10–15 μm at the widest region, in the area of the nucleus. The ovoid nucleus, about 15 μm in length, fills almost the entire width of the dark cell and contains small (0.5–1 μm) dense nucleoli. The nuclear envelope has a smooth contour. The perikaryonal cytoplasm contains prominent Golgi bodies, many free ribosomes, a small amount of rough endoplasmic reticulum and some large, irregularly shaped mitochondria with tubular cristae and a clear matrix. The apical cytoplasm contains membrane bounded dense granules, 0.15–0.35 μm in diameter, with a fine, particulate content of variable density; the smaller granules are more dense than the larger (Fig. 4). The granules are presumably responsible for the PAS positive staining of the apical cytoplasm of dark cells in light microscopic preparations, as shown in *Necturus* (Farbman and Yonkers, 1971). It should be noted, however, that the particulate content of the granules is apparent only after fixation in ice cold glutaraldehyde-OsO_4 mixture. Other fixatives reveal only the empty vacuoles described by other workers (Desgranges, 1965; Reutter, 1971). The basal regions of the dark cells extend into slender, "finger like" processes which interdigitate among the neuronal elements (Fig. 5). These processes contain prominent bundles of tonofilaments and scattered ribosomes and terminate by forming a hemidesmosomal attachment to the basal lamina. The processes also form desmosomal junctions with processes of adjacent dark cells, with vesicle containing processes of light cells and, more rarely, with basal cells.

The dimensions of the light cells are similar to those of the dark cells but the nucleus of the light cell is distinctive for three reasons. First, it contains a band of filamentous material often arranged as a closed ring (Fig. 2), but sometimes having the shape of the letter "c" in section. The filaments comprising the band are 10 nm in diameter. Second, nucleoli are rare. In our examination of several hundred electron micrographs taken from the nuclear region of taste buds, we have seen only three light cell nucleoli, 0.5–1 μm in diameter. Third, the light cell nucleus has an irregular contour, often with a broad and deep indentation.

The light cell has large mitochondria with tubular cristae and a clear matrix, a well developed Golgi system in the supranuclear region, and apically many membrane bounded vesicles (0.04–0.10 μm in diameter), irregular elongated sacs (up to 0.5 μm long), and tubular sacs expanded at the ends (Figs. 1, 4). Some light cells contain predominantly irregular, clear vesicles, and others smaller, more dense ones. Together, the vesicles, saccules and tubules appear to be part of a system of agranular endoplasmic reticulum. Small amounts of granular

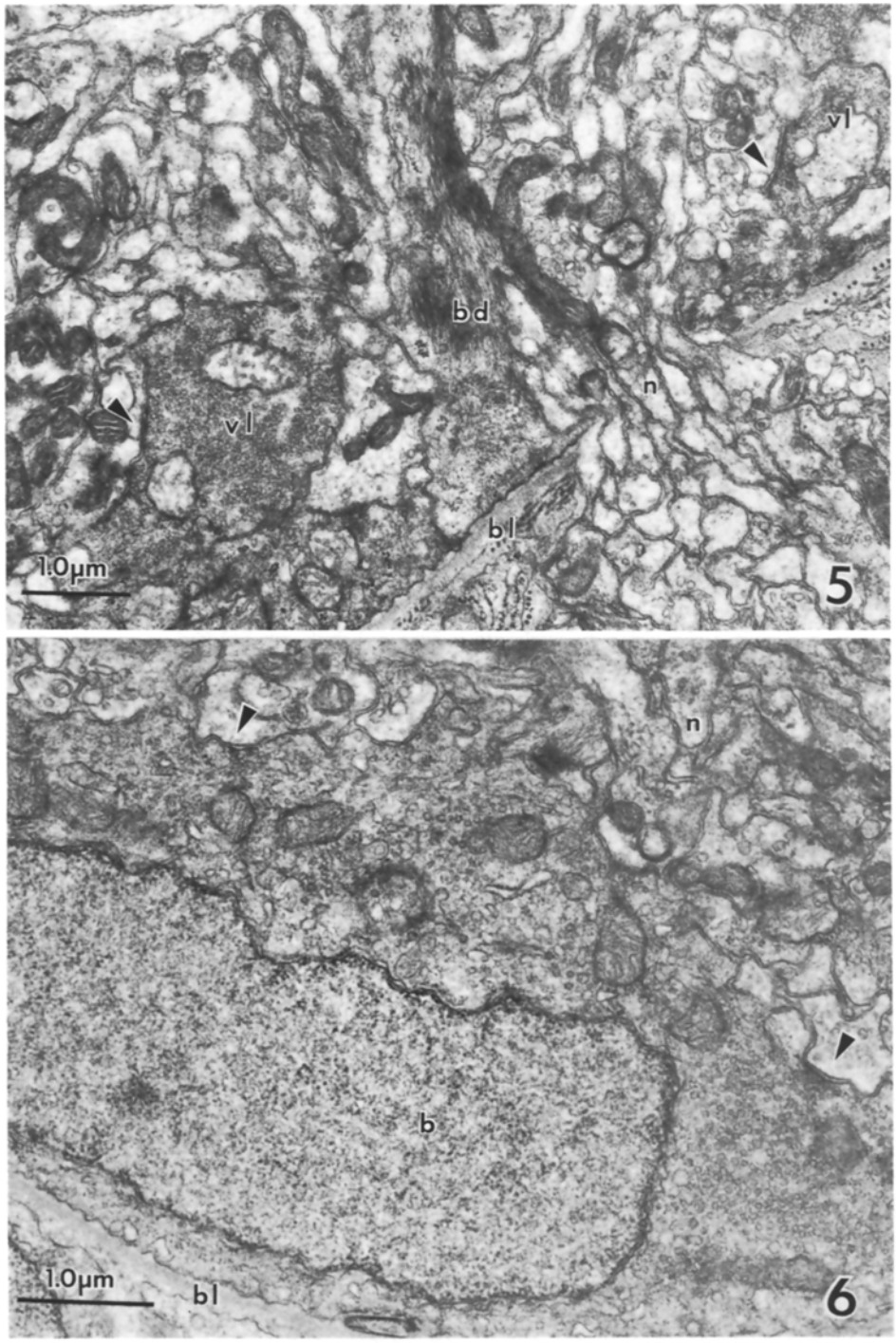


Fig. 5. Basal region of taste bud. *bd* basal process of dark cell. *vl* vesicle containing process of light cell. *n* unmyelinated nerve fibers. *bl* basal lamina. ▼ synapse. × 14,000

Fig. 6. Basal cell (*b*). *bl* basal lamina. *n* unmyelinated nerve fibers. ▼ synapse. × 19,000

endoplasmic reticulum, free ribosomes, and glycogen are found in the perinuclear region.

Basally the light cells terminate as vesicle containing processes which are frequently adjacent to the superior surface of a basal cell. This fact, derived from careful reconstructions of serial sections is contrary to the description by Reutter (1971) who judged the vesicle containing processes to be extensions of dark cells. The processes contain many small, clear vesicles 60 nm in diameter and mitochondria with clear matrix and tubular cristae (Fig. 5).

Three to five basal cells adjacent to and parallel with the basal lamina are present in each taste bud (Fig. 6). No hemidesmosome attachments to the basal lamina are seen at the inferior border of the cell. Instead, this surface has a ruffled appearance and is punctuated with many small pinocytotic vesicles (Fig. 6). The mitochondria in the basal cell, in contrast to those in the light and dark cells have shelf-like cristae. The basal cells contain ribosomes, occasional saccules of smooth endoplasmic reticulum, and glycogen particles. The nucleus is large and contains a nucleolus larger than those of light and dark cells and with an internal structure of granules and filaments. Small vesicles about 60 nm in diameter occur in large clusters, generally in the superior half of the cell. These vesicles are sometimes grouped near the periphery of the basal cell where there is contact with a nerve fiber, in configurations which resemble synapses (Fig. 6). A few dense-cored vesicles are present in the basal cell.

Between the basal cells, and the dark and light cells, large numbers of closely packed unmyelinated nerve fibers are seen. Their profiles contain only scattered microtubules, some glycogen particles, and mitochondria with a dense matrix and lengthwise shelf-like cristae (Figs. 5, 6).

A few structures resembling synapses are found in the basal region of the taste buds, specifically in two locations: (1) on the superior surface of the basal cells (Fig. 6); and (2) on the "vesicle containing process" or perikaryon of light cells (Fig. 5). In both cases a nerve fiber represents the postsynaptic part of the junction. In the presynaptic portion, synaptic-like vesicles of about 60 nm in diameter form clusters adjacent to the plasma membrane which is sometimes thickened.

Discussion

Some of the more interesting observations which have emerged from this study are the relative infrequency of nucleoli in light cells and the presence in their nuclei of a band of filaments in a ring or c-shaped configuration.

The scarcity of nucleoli in light cells is probably not a sampling problem since the corresponding structure in nearby dark cells was consistently observed. Absence of nucleoli and a relative paucity of cytoplasmic ribosomes suggests a low level of protein synthesis in light cells. This does not imply that the light cell with its many mitochondria and extensive smooth endoplasmic reticulum is metabolically inactive. The function of the light cell, however, is not yet apparent. Farbman and Yonkers (1971) have suggested that light cells are receptor cells and changes in their potential are in some way related to flux

across the membranes of smooth endoplasmic reticulum. Such an energy-dependent process would be consistent with the presence of many mitochondria in light cells.

Possibly associated with the scarcity of nucleoli in the light cells is the presence of intranuclear filamentous bands. Similar bands have been reported in amphibian oocytes treated with Actinomycin D (Lane, 1969). Treatment of oocytes with this drug disrupts nucleoli, inhibits RNA synthesis and results in the intranuclear accumulation of bundles of filaments. The filament bundles morphologically resemble those seen in the present study in light cells of catfish barbel taste buds, suggesting that light cells may also have a reduced level of RNA and protein synthesis. Curiously, bands of intranuclear filaments were not observed in light cells when taste buds were fixed in either buffered OsO_4 alone or in glutaraldehyde followed by postfixation in OsO_4. The filaments observed were only in those specimens fixed by: (1) Karnovsky's mixture followed by post-fixation in OsO_4, (2) buffered $KMnO_4$, and (3) at 0° C in the glutaraldehyde-OsO_4 mixture. The band of filaments corresponds to a lightly stained region in the nucleus seen with the light microscope in 1 μm sections stained with toluidine blue (cf. Lane, 1969). We agree with Lane's suggestion, that they probably represent protein filaments.

Granules in the apical cytoplasm of dark cells contain a moderately dense, finely particulate substance. This was observed only in those specimens fixed in the glutaraldehyde-OsO_4 mixture at 0° C. When tissues were fixed by any other means, our findings were consistent with those of others working with *Ictalurus* (e.g., Desgranges, 1965; Reutter, 1971) who observed clear vacuoles. The dark cells in the taste bud of catfish and most other vertebrates contain membrane-bounded secretory granules. In some species, the secretion granules stain with the periodic acid-Schiff reaction or the periodic acid-silver methenamine reaction (Nemetschek-Gansler and Ferner, 1964; Scalzi, 1967; Farbman and Yonkers, 1971). One exception is the taste bud of the rat fungiform papilla which apparently contains no identifiable secretory granules (Farbman, 1965, 1967). The function of these granules is not understood. They may provide the pore region with a glycoprotein-containing substance that maintains the chemical microenvironment. If the initial stimulus occurs on the exposed parts of receptor cells, a shift in chemical equilibrium in this region can be assumed.

The basal cell was first recognized as an independent cell type (i.e., not a stem cell for dark and light cells as Heidenhain, 1914, suggested) in fish taste buds by Hirata (1966) and has been described in the carp (Uga and Hama, 1967), in *Saccobranchus* (Rajbanshi and Tewari, 1968) as well as *Necturus* (Farbman and Yonkers, 1971). The paucity of desmosomes and virtual absence of hemidesmosomes on the basal cells suggests that they may not be of epithelial origin. Reutter (1971) has shown biogenic amines in basal cells by histochemical fluorescent methods. He concluded that they are neurally derived and may function as modifiers in the transmission of taste information from receptor cells to the central nervous system. We hypothesize that basal cells originate from the neural crest and migrate to the sites where taste buds will form.

The convergence of the "vesicle filled processes" of light cells towards basal cells and the close association between these two structures, suggests that a functional relationship may exist between them. We find no evidence of synaptic-

like junctions between these cell types, but membrane specializations in the form of desmosomes are present. The close association between the membranes of these cells and the presence of vesicles in both the basal cells and the vesicle filled process offers some support to Reutter's (1971) hypothesis that the basal cell receives the taste stimulus from light cells and synaptically transmits a modified impulse to the central nervous system. Synapse-like structures in the taste bud are often difficult to recognize, and were not numerous in our specimens. There is no definitive proof that these junctional specializations are true synapses, and, as Gray and Watkins (1965) point out in their study of rat taste buds, one should be wary of applying the designation "synaptic vesicle" with the implication that they contain a neurotransmitter.

References

Crisp, M., Lowe, G.A., Laverack, M.S.: On the ultrastructure and permeability of taste buds of the marine teleost, *Ciliata mustela*. Tiss. and Cell **7**, 191–202 (1975)

Desgranges, M.J.: Sur l'existence de plusieurs types de cellules sensorielles dans les bourgeons du gout des barbillons du Poisson-chat. C. R. Acad. Sc. (Paris) **261**, 1095–1106 (1965)

Desgranges, M.J.: Sur la double innervation des cellules sensorielles des bourgeons du gout des barbillons du Poisson-chat. C. R. Acad. Sci. (Paris) Ser. D **263**, 1103–1106 (1966)

Desgranges, M.J.: Sur les bourgeons du gout du Poisson-chat *Ictalurus melas:* Ultrastructures des cellules basales. C. R. Acad. Sci. (Paris) Ser. D **272**, 1814–1817 (1972)

Farbman, A.I.: Fine structure of the taste bud. J. Ultrastruct. Res. **12**, 328–350 (1965)

Farbman, A.I.: Structure of chemoreceptors. In: Symposium on Foods; Physiology and Chemistry of Flavors. (H.W. Schultz, E.A. Day, L.M. Libbey, eds.) pp. 25–51. Avi Publishing Co. 1967

Farbman, A.I., Yonkers, J.D.: Fine structure of the taste bud in the mud puppy, *Necturus maculosus*. Amer. J. Anat. **131**, 353–370 (1971)

Gray, E.G., Watkins, K.C.: Electron microscopy of taste buds in the rat. Z. Zellforsch. **66**, 583–595 (1965)

Heidenhain, M.: Über die Sinnesfelder und die Geschmacksknospen der Papilla foliata des Kaninchens. Arch. mikr. Anat. **85**, 365–479 (1914)

Hirata, V.: Fine structure of terminal buds on the barbels of some fishes. Arch. Histol. Jap. **26**, 507–523 (1966)

Karnovsky, M.J.: A formaldehyde-glutaraldehyde fixative of high osmolality for use in electron microscopy. J. Cell Biol. **27**, 137a (1965)

Lane, N.J.: Intranuclear fibrillar bodies in actinomycin D-treated oocytes. J. Cell Biol. **40**, 286–291 (1969)

Nemetschek-Gansler, H., Ferner, H.: Über die Ultrastruktur der Geschmacksknospen. Z. Zellforsch. **63**, 155–178 (1964)

Olmsted, J.M.D.: The results of cutting the seventh cranial nerve in *Ameiurus nebulosus* (Leseur). J. exp. Zool. **31**, 369–401 (1920)

Rajbanshi, V.K., Tewari, H.B.: Structure of the taste bud of *Saccobranchus fossilis*. Z. Biol. **116**, 22–28 (1968)

Reutter, K.: Die Geschmacksknospen des Zwergwelses *Ameiurus nebulosus* (Leseur). Morphologische und histochemische Untersuchungen. Z. Zellforsch. **120**, 280–308 (1971)

Scalzi, H.A.: The cytoarchitecture of gustatory receptors from the rabbit foliate papillae. Z. Zellforsch. **80**, 413–435 (1967)

Stensaas, L.J.: The fine structure of fungiform papillae and epithelium of the tongue of a South American toad, *Calyptocephalella gayi*. Amer. J. Anat. **131**, 443–462 (1971)

Uga, S., Hama, K.: Electronmicroscopic studies on the synaptic region of the taste organ of carps and frogs. J. Electron Micros. **16**, 269–277 (1967)

Welsch, U., Storch, V.: Die Feinstruktur der Geschmacksknospen von Welsen [*Clarias batrachus* (L.) und *Kryptopteros bicirrhis* (Cuvier et Valenciennes)]. Z. Zellforsch. **100**, 552–559 (1969)

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