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Heterogeneity of fish taste bud ultrastructure as demonstrated in the holosteans *Amia calva* and *Lepisosteus oculatus*

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Taste buds are the peripheral sensory organs of the gustatory system. They occur in all taxa of vertebrates and are pear-shaped intra-epithelial organs of about 80 µm height and 50 µm width. Taste buds mainly consist of specialized epithelial cells, which synapse at their bases and therefore are secondary sensory cells. Taste buds have been described based on studies of teleostean species, but it turned out that the ultrastructure of teleostean taste buds may differ between distinct systematic groups and that this description is not representative of those taste buds in other main taxa of fishes, such as selachians, holosteans and dipnoans. Furthermore, it is not known how variable the micromorphologies of non-teleostean taste buds are. For this reason the taste buds of two holosteans, *Lepisosteus oculatus* and *Amia calva*, were investigated and compared. While in both species the taste buds are of the same shapes and sizes, the cellular components of their sensory epithelia differ: in *Lepisosteus* taste buds comprise two types of elongated light cells and one type of dark cells. In contrast, *Amia* taste buds contain only one type of light, but two types of dark elongated cells. Afferent synapses are common in the buds of both species, efferent synapses occur only in *Lepisosteus* taste buds. These differences show that even in the small group of holostean fishes the taste buds are differently organized. Consequently, a representative type of fish taste buds does not exist.

Keywords: taste bud; ultrastructure; cell types; vertebrates; fishes; systematics

1. INTRODUCTION

In fishes, as in other vertebrates, the taste buds are the peripheral sense organs of the gustatory system. They occur in the epithelia of the oropharyngo-branchial cavity and in some species also in the epidermis. Taste buds receive their input from tastants and transmit the information to the central nervous system via the cranial nerves VII (facial nerve), IX (glossopharyngeal nerve) and X (vagal nerve). These nerves terminate in enlarged lobes of the medulla oblongata, the facial lobe and the (glossopharyngeal-) vagal lobe. From there, higher and lower centres of the brain are reached (Finger & Morita 1985).

Taste buds are pear-shaped organs that consist of up to 100 specialized epithelial, mostly elongated cells. At their bases the sensory cells are synaptically connected to afferent nerve fibres. So far, as revealed by developmental studies, taste buds contain secondary sensory cells (Reutter & Witt 1993; Sorensen & Caprio 1998).

In fishes, taste buds enable the animal to identify food by detecting distinct chemical substances on a short distance (Kasumyan 1997). In contrast, the fish's olfactory system is known to be its long-distance chemical sense, which enables the fish to find food and to communicate with other individuals (Jones 1992). A third chemosensory system is represented peripherally by the solitary chemosensory cells (Kotrschal 1991; Whitear 1992).

Fish taste buds consist of several cellular components. They comprise the elongated light and dark cells that build the organ's sensory epithelium. Its cells terminate apically with microvillar structures, the receptor villi, which build the receptor area. At their bases the elongated cells are synaptically connected to the organ's nerve fibre plexus. Below this structure the basal cells are located. The organ's circumference consists of marginal cells, which are non-specialized epithelial cells. As a rule, taste buds sit atop a dermal papilla (for reviews, see Kapoor *et al.* 1975; Jakubowski & Whitear 1990; Reutter 1978, 1986; Reutter & Witt 1993; Sorensen & Caprio 1998). Hitherto, taste bud morphology in fishes seemed to follow one bauplan. But then ultrastructural investigations carried out in non-teleostean fishes revealed clearly that taste buds differ within the main vertebrate taxa (Reutter & Witt 1993), and even in different systematic groups of fishes the taste buds do not follow only one structural design (Reutter & Witt 1999). Now, this turns out to be true especially for the small taxon of holosteans for which we have only little information about taste bud ultrastructure (Boudriot & Reutter 1998; Reutter & Witt 1999). This might be due to the fact that holosteans are not of great economic value and interest, they are not bred in farms and are not laboratory animals. On the contrary, in fish taste bud ultrastructure research, the ancient and small taxon of the holosteans is of distinct

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Table 1. *Comparison of the main taste bud (TB) components of fishes which belong to different systematic groups*

	Selachii		Holostei		Teleostei		Dipnoi
	Scyliorhinus	*Raja*	*Lepisosteus*	*Amia*	*Ameiurus* (A) *Silurus* (S)	*Astyanax*	*Neoceratodus*
TB size (height/width, μm)	50/30	no TBs	65/45	65/45	55/35 (A) 80/50 (S)	60/35	100/80
TB position		(single and	+	[+][b]	+	+	+
in epidermal hillock	+[a]	groups of soli-	−	(+)[c]	+	+	(+)
in flat epithelium	−[d]	tary chemo-	+	+	+	+	+
on top of dermal papilla	−	sensory cells)					
cell types							
light cell	one type	—	two types	one type	one type	two	one type
dark cell	two types	—	one type	one (two) type	one type	one	one type
marginal cell	+	—	+	+	+	+	+
basal cell	+	—	+	+	+	+	+
location	outside TB base	—	at TB base	at TB base	at TB base	at TB base	at TB base
with spines	−	—	−	(+)	+ (A); + (S)	(+)	−
basal nerve fibre plexus	small	—	small	small	large	small	small
ascending nerve fibres	−	—	−	−	−	−	+
synapses							
afferent	+	—	+	+	+	+	+
efferent	−		+	−	(+)(A); − (S)	−	+
references	Reutter 1994; Whitear & Moate 1994*a*	Whitear & Moate 1994*b*	Reutter & Witt 1996	Boudriot & Reutter 1998	Desgranges 1966; Reutter 1986,1987; Royer & Kinnamon 1996	Boudriot & Reutter 2000	Reutter 1991

[a] +, yes.
[b] [+], yes, but in low hillock.
[c] (+), yes, but seldom.
[d] −, no.

interest, and raises the following questions. Does there exist a taste bud type that is representative of all holostean fishes? And, are there striking differences between the taste buds of *Lepisosteus oculatus* and *Amia calva*, the two species that were compared in this study?

2. RESULTS AND CONCLUSIONS

By reasons of comparability the methods used in this study were the same as in our former studies.

The fishes investigated here were gifts from Dr John Caprio, Louisiana State University, Baton Rouge, LA. They originate from the Fisheries Department at Louisiana State University (*L. oculatus*) and the aquarium in New Orleans (*A. calva*). The ethical regulations published in the Declaration of Helsinki (1995) were respected. After deep 3-aminobenzoic acid ethyl ester (Tricane, MS 222; Sigma–Aldrich) anaesthesia of the fish, the tissues were excised and fixed in 0.1 M phosphate buffer (pH 7.2) containing 2.5% glutaraldehyde or 2% paraformaldehyde. After osmication, the specimens were embedded in Araldite. Ultrathin sections were stained with uranyl acetate and lead citrate.

Both holostean fishes, *Amia* and *Lepisosteus* possess taste buds that seem to be organized similarly to the organs of other fishes. They occur in the epithelia of the oropharyngo-branchial cavity, are ovoid or pear-shaped and sit atop a dermal papilla. In both species the taste buds are relatively small and comprise about 50–60 cells. Most of them are elongated cells, the light (electron-lucent) cells and the dark (electron-dense) cells. Furthermore, there are marginal cells, basal cells and a nerve fibre plexus, which is situated between the bases of the elongated cells and the basal cells.

Looking more closely at the cellular components of *Lepisosteus* and *Amia* taste buds it is obvious that they are distinctly different from each other (table 1 and figure 1). A *Lepisosteus* taste bud contains two subtypes of light elongated cells, one of which apically contributes to the organ's receptor area with one large and cone-like receptor villus, whereas the other subtype possesses several small undivided receptor villi. In contrast, an *Amia* taste bud contains only one type of elongated light cell that ends in one large receptor villus. In both species the dark cells also differ. *Lepisosteus* taste buds possess one type of elongated dark cell. Apically it contains intensely stained secretory vesicles and terminates with numerous small and sometimes divided receptor villi. *Amia* possibly has two subtypes of dark cells: both of them are also rich in electron-dense secretory vesicles, but terminate either with one big and irregularly lobed receptor villus or with several small and divided receptor villi. Furthermore, in *Lepisosteus* taste buds centrioles occur exclusively in the apical cytoplasm of the light cells; in *Amia* centrioles were

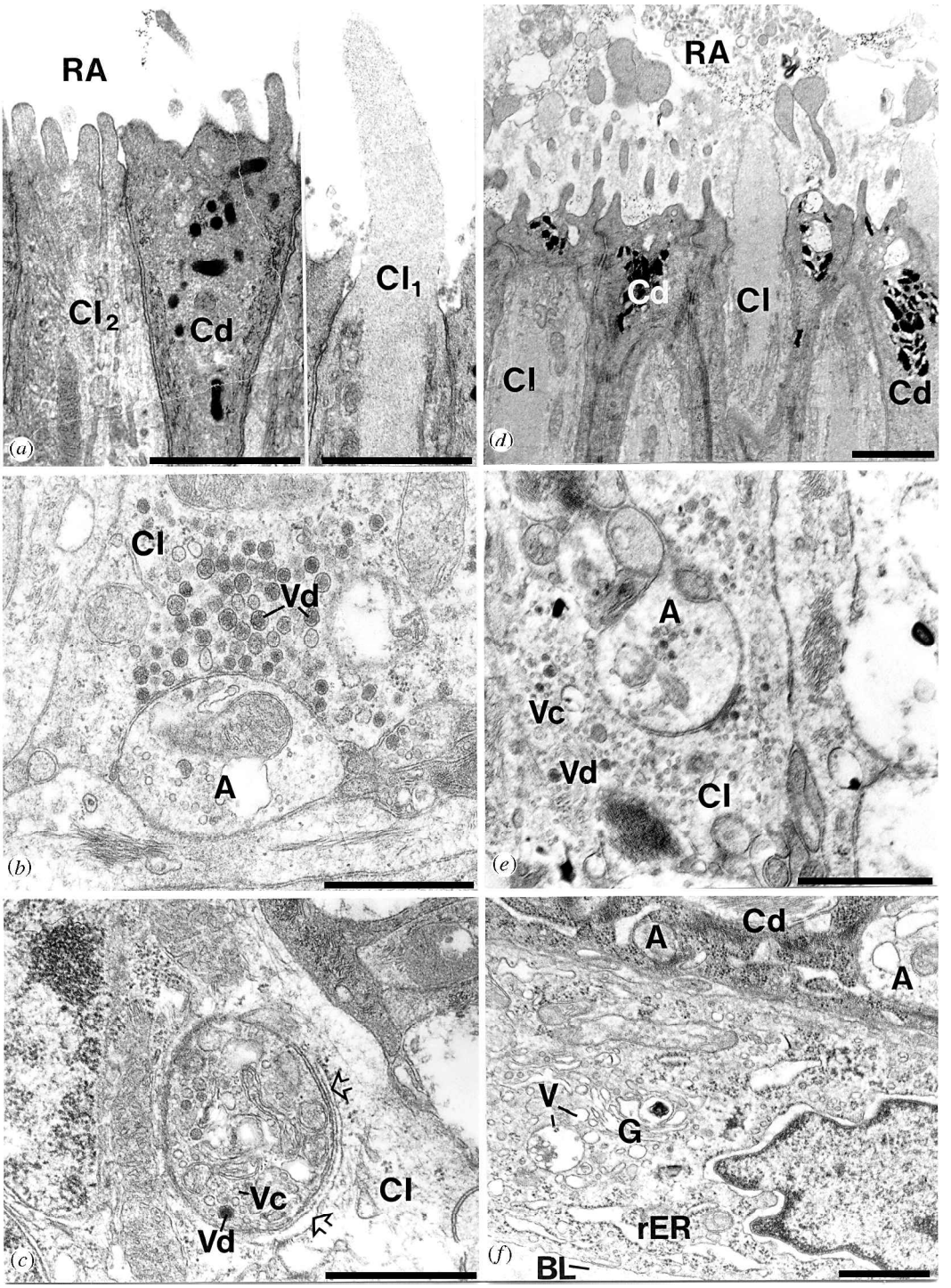

Figure 1. Transmission electron micrographs of longitudinally cut holostean taste buds. (*a–c*) *L. oculatus*, (*d–f*) *A. calva*. (*a*,*d*) Apical parts of elongated taste bud cells. In *Lepisosteus*, two types of light cells (Cl_1 and Cl_2) and one type of dark cell (Cd) occur; in *Amia*, one type of light cell and at least one type of dark cell. Note the different profiles of the receptor villi within the receptor area (RA). (*b*,*e*) Afferent synapses between light cells (presynaptic sides) and axons (A) of the taste bud's nerve fibre plexus. In *Lepisosteus*, the synapse is rich in dense-cored vesicles (Vd); in *Amia*, there are more small clear vesicles (Vc) than dense-cored ones. (*c*) Efferent synapse situated between an axon (presynaptic side) and a light cell. The axon contains some small clear vesicles (Vc) and a few dense-cored vesicles (Vd). Note the subsynaptic cisterna (open arrows) on the light cell's side. (*f*) Perinuclear region of a basal cell, which rests on the basal lamina (BL). Apically, the cell is covered by the structures of the nerve fibre plexus: axons and the basal processes of dark (and light) cells. Note the cell's richness in organelles, such as Golgi (G) complexes, rough endoplasmic reticulum (rER) and vesicles (V) of different sizes. Bars, 1 μm throughout.

found only in the apical cytoplasm of the dark cells. In both species afferent synapses are situated between the bases of the elongated cells (presynaptic sides) and the axons of the nerve fibre plexus, and between the basal cells (presynaptic sides) and the axons. Some rare efferent synapses were seen only in *Lepisosteus* taste buds. They are situated between an axon (presynaptic side) and a light cell. In comparison with the teleostean taste bud, in both

holostean fishes the organ's nerve fibre plexus is relatively small. It is situated between the bases of the elongated cells and the basal cells.

Comparing the taste buds of *Amia* and *Lepisosteus* with the taste buds as they were described in the other main taxa of fishes (table 1) like Selachii Teleostei and Dipnoi, it is clear that the taste buds of both holosteans do not fit well into the design of taste buds of another group of fishes, they have characteristics of their own. What is more, both holostean species have taste buds that differ from each other: for instance, with respect to their cellular composition and their basal cells, which may have microvillar spines or not (for references see table 1).

The result of this comparative study is surprising. We did not expect that in the small group of holosteans (including three families with about 20 species) taste bud ultrastructure would vary, even between the Lepisosteidae (seven species) and Amiidae (one species), and would not follow one general design. Unfortunately, the ultrastructure of taste buds of the third holostean family, Polypteridae, are not known yet. But, probably there exists no common 'holostean taste bud type'. The peripheral taste organs in other main taxa of fishes vary, too: in the selachians true taste buds were described in *Scyliorhinus* (Reutter 1994; Whitear & Moate 1994*a*); they are lacking in *Raja* (although masses of single and aggregated solitary chemosensory cells are present; Whitear & Moate 1994*b*). Teleostean taste buds were thought to be representative of all kinds of fishes. But there is increasing evidence that also in this big group of fishes taste buds differ. 'Normal' teleostean taste buds are equipped with one type of light cell and one type of dark cell (see references in Reutter & Witt 1999), but there exist also taste buds that possess one type of dark cell and two types of light cells (Cyprinidae: *Danio*, Hansen *et al.* 1999; Characidae, Boudriot & Reutter 2000). And, obviously also the dipnoans have their own type of taste buds (Reutter 1991). This indicates that fish taste bud ultrastructure is taxon related and also might be species specific. Thus, we hypothesize that fish taste buds do not belong to a common 'fish taste bud type', as it does not exist.

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