C(S)

Ontogeny of the Solitary Chemosensory Cells in the Zebrafish, *Danio rerio*

Kurt Kotrschal, Wolf-Dietrich Krautgartner[1] and Anne Hansen[2]

Zoologisches Institut der Universität Wien und Konrad Lorenz Forschungsstelle, A-4645 Grünau 11,
[1]Zoologisches Institut der Universität Salzburg, Hellbrunnerstraße 34 A-5020 Salzburg, Austria and
[2]Zoologisches Institut und Museum, Martin-Luther-King-Platz 3, D-20146 Hamburg, Germany

Correspondence to be sent to: Kurt Kotrschal, Konrad Lorenz Forschungsstelle, A-4645 Grünau 11, Austria

Abstract

Secondary epidermal solitary chemosensory cells (SCCs) are widespread among the primary aquatic vertebrates. They resemble taste bud sensory cells in fine structure and may be innervated from facial or spinal nerves. According to previous studies, SCCs may constitute a water sampling system in the contexts of predator avoidance, habitat recognition and, in some cases, finding food. By quantitative scanning (SEM) and transmission electron microscopy (TEM) in 60 specimens (57 SEM, 3 TEM) of 16 developmental stages, from pre-hatchlings to adults, we describe the ontogenetic development of SCC densities and shapes of sensory apices in the zebrafish, *Danio rerio*. This is put into perspective with the ontogeny of external taste buds. Just prior to hatching, 3 days after fertilization (3d AF), sensory apices of SCCs penetrate between the squamous epidermal cells, whereas taste bud pores only appear at the onset of exogenous feeding (5d AF). SCC densities increase sharply from hatching shortly after metamorphosis (25d AF) up to 6×10^3 per mm^2 on the head and remain relatively constant in density thereafter. Conservatively estimated, there may be $\sim 3.2 \times 10^5$ SCCs on the head and 1×10^6 SCCs on the entire body surfaces of a zebrafish 180d AF. SCCs are spread evenly, but are 2- to 5-fold higher in density along the head than along the body. Sensory apices are brush-like in hatchlings and early juveniles, but tend to consist of a single villus in the adults. This ontogenetic change of SCC apices parallels the evolutionary change from 'oligovillous' cells in lampreys and elasmobranchs to the 'monovillous' SCCs in the advanced actinopterygian teleosts. **Chem. Senses 22: 111–118, 1997.**

Introduction

Aside from taste buds, the epidermis of primary aquatic vertebrates, notably fish, is plentifully equipped with singular, spindle-shaped, secondary sensory cells, the so-called solitary chemosensory cells (SCC; Whitear, 1965, 1992; Kotrschal, 1991, 1996). Such cells have been found in lampreys ('oligovillous cells'; Whitear and Lane, 1983; Baatrup, 1984a,b), skate (Whitear and Moate, 1994), all groups of gnathostome fish (Kotrschal, 1991) and also some anuran tadpoles (Whitear, 1976). In a number of freshwater teleosts, distribution over the body surface was found to be relativly even, with higher densities on the head than at the body. Densities of SCCs varied between 200 per mm^2 (in the neon tetra, *Hypessobrycon innesi*) and $<4 \times 10^3$ SCCs per mm^2 (in the roach, *Rutilus rutilus*; Kotrschal, 1992). The highest SCC densities of up to 1.0×10^5 per mm^2 (Kotrschal *et al.*, 1984) were found in a peculiar, chemoreceptive dorsal fin of rocklings (Gadidae, Teleostei). Electrophysiological recordings from this organ showed a narrowly tuned chemo-

responsiveness to only a few natural stimuli, such as skin surface washes from other fish or dilutions of bile, but not to amino acids (Peters, *et al.*, 1991; Kotrschal *et al.*, 1996).

Because of their distribution, their specific stimuli and the behavioral responses elicited, SCCs were found to be distinct from taste buds (Kotrschal *et al.*, 1993a,b; Essler and Kotrschal, 1994). Diluted fish mucus caused alert responses in intact rocklings, but had no effect when the chemosensory dorsal fin was removed (Kotrschal *et al.*, 1989). Also, finding prey was not impaired in these fish (Whitear and Kotrschal, 1988). Therefore, it was hypo- thesized that SCCs form a bulk-water sampling system involved mainly in monitoring the presence of other fish upstream, including predators (Kotrschal, 1996), but less in finding food. However, these results from rocklings may not be representative for all SCC systems. In sea robins (Triglidae, Teleostei), for example, pectoral fin rays are used to probe the substrate. Their SCC-like cells are spinally innervated and probably serve to discriminate food (Silver and Finger, 1984).

With the present paper we aim to describe the quantitative distribution of SCCs along the body surface in zebrafish from fertilization to 180 days thereafter. Such a study is relevant with respect to the following hypotheses and predictions:

1. If SCCs are indeed involved in predator avoidance, this system should be functional as soon as possible, because predation pressure is highest in the smallest fish. SCC apices therefore should penetrate between the squamous epidermal cells at hatching, 3 days after fertilization. In contrast, taste buds may appear later, because they serve to discriminate food and are therefore not needed before the onset of exogenous feeding, which is 2 days after hatching.
2. An investigation of the postlarval growth of the peripheral gustatory system in the Channel catfish (*Ictalurus punctatus*; Finger *et al.*, 1991) revealed pronounced allometric shifts of the taste system during growth. For example, absolute taste bud numbers increased, even though relative (to unit area) taste bud densities decreased. Also, the number of cells per taste bud increased and increasing numbers of taste cells converged onto a single nerve fiber. Therefore, the system may improve during growth with respect to spatial acuity and threshold discrimination. We here investigate whether possible ontogenetic shifts in the zebrafish SCC system allow similar functional predictions. Increasing SCC numbers, for example, may indicate decreasing threshold, given that the innervating nerve fibers increase at a slower rate than the SCCs. Spatial acuity of the system may increase with SCC density.
3. As no ancestral species were found with either taste buds or SCCs alone, the question of the evolutionary relationship between both systems cannot be answered conclusively. Either taste buds may be seen as aggregated SCCs (Whitear, 1971) or SCCs may be dissociated taste buds. The earlier ontogenetic appearance of SCCs or taste buds may at least reveal a hint on the ancestral form of structural organization—whether it be SCCs or taste buds. Also, taste bud sensory cells are exclusively monovillous. The ontogenetic and phylogenetic distribution of oligovillous apices may therefore provide another hint on the ancestral or derived state of SCCs versus taste buds.

Materials and methods

Zebrafish were obtained from our own breeding stock (AH, Zoological Institute, University of Hamburg), which is kept in accordance with Westerfield (1993). Adults of both sexes were kept in 50 or 100 l tanks on a 14 h light:10 h dark cycle at a water temperature of 26°C (Hansen and Zeiske, 1993). Fertilized eggs were collected and kept in Petri dishes until hatching. Larvae were reared in small glass containers. Embryos, larvae and adult fish were anaesthetized with MS 222 (~0.03%) and fixed by immersion in 5% glutaraldehyde in 0.05 M phosphate buffer (pH 7.2). Embryos (1–3 days after fertilization) were dechorionated before fixation. After rinsing in phosphate buffer, specimens were dehydrated in a graded series of acetone, critical-point dried in CO_2 and sputtered with gold. TEM preparation was according to standard procedures. Larvae fixed in Karnovsky's fixative were rinsed and dehydrated in a graded series of alcohol and embedded in epon resin. The heads of three larvae were cut at 50 nm and examined under a Philips 300 TE microscope.

SCC sensory apices were counted in series of standard areas of 3170 mm^2, on the screen of a Cambridge stereoscan at low acceleration voltage. At least five of these frames were distributed along transects, from rostral to caudal along both sides of the forehead. Also, at least five areas were sampled along the sides of the mid-body, dorsal and caudal to the lateral line. Fifty-seven specimens of 16 developmental stages, from day 1 after fertilization to day 180,

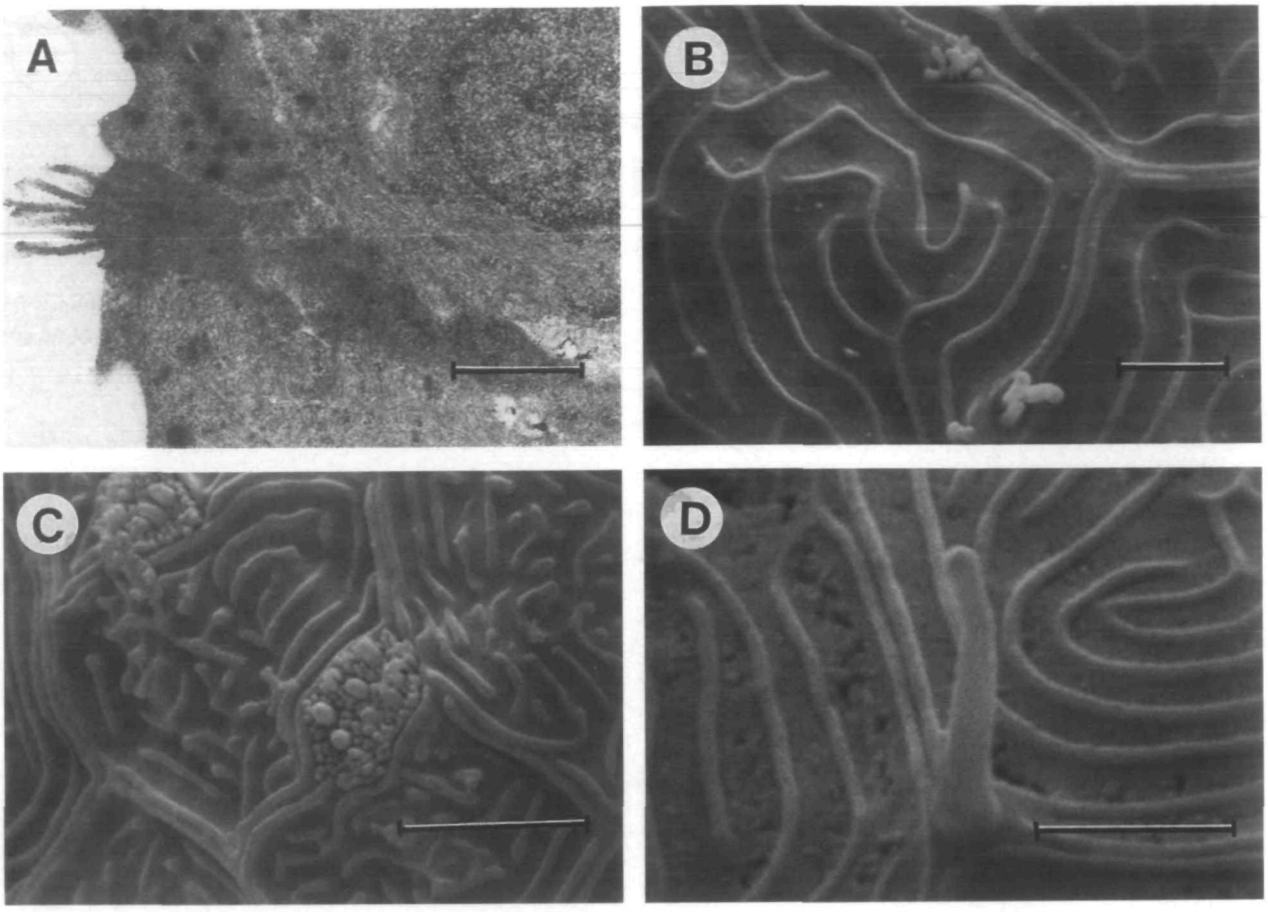

Figure 1 **(A)** Transmission electron micrograph of an 'oligovillous' apex of a solitary chemosensory cell penetrating the squamous epidermal cells in a zebrafish embryo 3 days after fertilization, shortly before hatching. Note that separate cytoskeletal elements (fibrillous cores) extend into the cytoplasm of each villus. **(B)** Scanning electron micrograph of the apices of solitary chemosensory cells in a zebrafish larva 5 days after fertilization. Two brushlets of sensory apices emerge between the borders of squamous epidermal cells, one showing ~9 villi (top) and the other 3 (bottom). **(C)** Scanning electron micrograph of two newly emerged taste bud pores (center and top left) in a zebrafish larva at the onset of exogenous feeding, 5 days after fertilization. **(D)** Scanning electron micrograph of the apex of a solitary chemosensory cell in an adult zebrafish, 180 days after fertilization.

were investigated. As no trend in SCC density within these transects was found, the mean number of SCC apices per average sampling area was calculated in each specimen, for head and body separately. Stage means were calculated based on individual means (Table 1).

Head dimensions of specimens prepared for SEM were measured with the aid of a binocular, equipped with camera lucida to the nearest 0.1 mm. Head area was estimated with a simple cone-model using the head diameter as the base (mean of width and height) and head length (from the posterior opercular rim to the tip of the snout) as cone height. Despite the fact that counts of 15 test areas by the three authors resulted in virtually the same results (means differed <10% and showed the same coefficient of variation), counts were performed by only one of us (W.D.K.) to ensure constant results.

This method of counting results in conservative estimates, because some of the SCCs may elude detection if their apices are broken during the SEM preparation procedure. To qualify as an SCC apex, shape, size and position between the squamous epidermal cell borders were considered. Criteria were finger- to brush-like membraneous extensions, 0.5–5 mm in length, protruding between the borders of squamous epidermal cells. Doubtful structures were excluded from the counts. Areas with damaged or mucus-covered epidermis were not considered, causing lower-than-planned sample sizes in a few specimens. A certain unavoidable parallax during scanning of the skin surfaces may have slightly increased the area sampled in some cases.

Results

Individual apices of SCCs in zebrafish may assume a continuum of shapes, from brushlets of 2–7 small, equally sized villi, 0.5–1 mm in length, which emerge independently

Table 1 Developmental stages (days after fertilization) of zebrafish and number of specimens investigated for densities of solitary chemosensory cells

Stage days	Number of specimens	Head (mean ± SD)	Body (mean ± SD)	Life history events
1	4	0	0	
2	4	0	0	
3	4	530 ± 78	383 ± 78	hatching
4	4	557 ± 83	383 ± 143	
5	2	1004 ± 0	1195 ± 456	onset of exogenous feeding, external taste buds appear
7	4	404 ± 120	64 ± 52	
10	3	1166 ± 377	1009 ± 180	
13	4	3421 ± 1247	1770 ± 198	onset of metamorphosis
20	3	1297 ± 521	1754 ± 1244	
25	4	6009 ± 889	2129 ± 227	
35	4	2958 ± 858	431 ± 253	
45	4	6904 ± 1672	1068 ± 909	
50	1	6051	–	
75	4	4520 ± 428	–	onset of sexual maturity
120	4	2551 ± 1453	1196 ± 138	
180	4	5772 ± 1954	2296 ± 546	fully grown adults
Totals				
16 stages	57 individuals			

–: no data because of damaged skin surface.

from each others from the distal cell surface, to single finger-like villi, which may exceed 3 μm in length (Figures 1A,B,D and 3). However, it is not clear whether these differences represent developmental states of individual SCCs or whether the SCC apices change from brush-like to single-villous in the ontogeny of individual fish through a succession of cells. Alternatively, the diverse shapes of apices may represent fixation snapshots of the dynamic apex of individual SCCs. It seems, however, that in the youngest stages (3–5 days after fertilization; d AF) in which SCCs have been found only tiny, often brush-like apices are present (Figure 1A,B), whereas adult zebrafish carry mainly single-villous apices (Figure 1D). This would indicate a change of the apex with ontogeny.

The first SCC apices were found by TEM 3d AF, just prior to hatching (Figure 1A), whereas external taste buds were not found before 5d AF, shortly before the onset of exogenous feeding (Figure 1C). Also, Anlagen of taste buds and, possibly, of neuromasts were found within the epidermis by TEM (not shown). Occasionally, goblet cells had already opened towards the surface. The dark sub-surface vesicles within the squamous epidermal cells may contain hatching enzyme (Figure 1A). By SEM, no

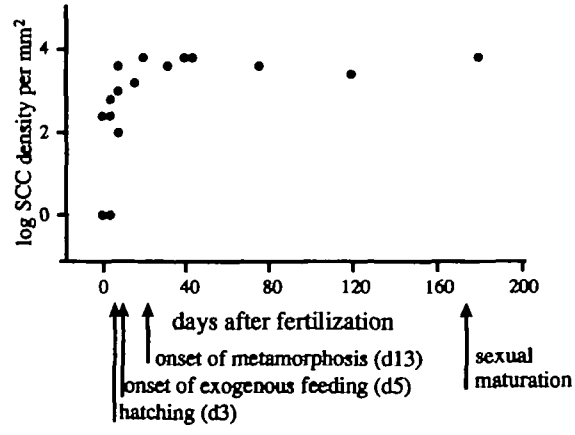

Figure 2 Logarithmic representation of the development of densities of solitary chemosensory cell apices per mm^2 on the head of zebrafish, 1–180 days after fertilization (*x*-axis). Each of the 16 developmental stages is based on counts in 1–4 individuals (cf. Table 1 for sample size and variation).

SCCs were found on embryos peeled out of the eggshell (Table 1), which may be due to fixed secretion, which obstructed the view at the body surfaces.

The density of SCCs per unit area increases positively allometric with the head surface (Figure 2). There is a sharp increase from hatching to 25d AF, from a few hundred SCCs

up to >7 × 10^3 per mm^2 (Table 1) in individual fish (on average up to 4 × 10^3 per mm^2; Figure 2). Conservatively estimated, there may be 3.2 × 10^5 SCCs on the head of a 180d AF individual and a total of >1 × 10^6 SCCs on the entire body surface. Throughout development, SCCs on the head exceed those on the body by 2- to 5-fold in density (Table 1). Solitary cell apices seem to penetrate at the same time on the head and on the body. In contrast to previous findings (Kotrschal, 1992), densities of SCCs in an area of 120 × 120 mm^2 around free neuromasts on the head were no higher than in adjoining control areas in specimens 45 and 75d AF (n = 10 and 9 respectively).

Discussion

In zebrafish, SCC densities at the body surface are within the range of a few hundreds to a few thousands per mm^2 estimated in other fish, notably cyprinids (Kotrschal, 1992). Towards sexual maturity (past 180d AF), however, up to 7 × 10^3 SCC apices per mm^2 were counted in zebrafish, which exceeds the densities of all other generalized SCC systems considered until now (Kotrschal, 1995). However, it is still well below the 1 × 10^5 per mm^2 counted on the specialized anterior dorsal fin of rocklings (Kotrschal *et al.*, 1984).

As in a few other teleosts (Kotrschal, 1992), SCCs in zebrafish are relatively evenly distributed over the body surface. However, in all fishes densities were found to be highest on the head. This agrees with the functional flow-dependence of SCCs found in rocklings (Peters *et al.*, 1991; Kotrschal *et al.*, 1993a). Due to body shape, flow velocity along the surface of a swimming fish is lowest at the tip of the snout and accelerates along the head surface towards a maximum at the greatest lateral extension of the body. Towards the tail, the body tapers and flow velocities decrease. Accordingly, SCC densities were found to be rather low at the tip of the snout and highest at the sides of the head, decreasing towards the tail. Local SCC densities therefore seem to reflect local flow velocities, and thus be in proportion with the relative thickness of the boundary layer along the body surface (Kotrschal *et al.*, 1993a). The faster the laminar flow over a body surface, the thinner the boundary layer and the faster the diffusion of stimulus molecules to the membrane receptors of the SCC apex. Indeed, responses from the rockling dorsal fin could only be recorded from the actively sampling (undulating) fin (Peters *et al.*, 1991), suggesting that flow is a crucial factor for chemosensory perception. On these grounds it was hypothesized (Kotrschal, 1996) that increased swimming speed and zig-zagging in aroused fish may serve to improve SCC sampling (Essler and Kotrschal, 1994; Kotrschal and Essler, 1995).

If SCCs were involved in predator avoidance (Kotrschal, 1991, 1995, 1996), one could predict that SCC densities would vary with general predation pressure. Therefore, SCC densities should be particularly high in small fish. The present results support this prediction.

1. SCCs appear at hatching, 2 days earlier than taste buds. The fact that taste buds are only present at the onset of exogenous feeding underlines their role in finding food. Conversely, the earlier appearance of SCCs may indicate that these are not involved in feeding.
2. SCC density increases sharply and positively allometric relative to the body surface up to 25d AF, when fish are smallest, inexperienced and thus most vulnerable to predation (Figure 2). As SCCs probably form a bulk-water sampling system, enhanced spatial resolution by increasing SCC densities may be less important than a possible decrease in thresholds. In analogy to the retina, the chance of a hit by a single stimulus molecule (which can only penetrate the boundary layer by diffusion, on a Brownian path) may depend on the amount and density of available receptive membranes.

Chemical stimuli may alert individuals to the upstream presence of other fish over greater distances than vision. Particularly in freshwater, vision is easily obstructed by turbidity, vegetation, etc. The involvement of chemosenses in predator detection in post-hatchlings would be the more useful, as the visual reactive distances of fishes are short early in ontogeny (Wanzenböck *et al.*, 1996).

The olfactory system even precedes SCCs. Olfactory axons enter the forebrain 24 h AF and the olfactory pit opens 32–36 h AF, complete with developed olfactory knobs and cilia (Hansen and Zeiske, 1993). As SCCs seemingly penetrate the epidermal surface around hatching (Figure 1A), this would allow cross-talk between olfactory and SCC input to occur in order to generate specific behavioral responses (proposed by Essler and Kotrschal, 1994; Kotrschal, 1996; Kotrschal and Finger, 1996). It may be assumed that SCCs become functional upon penetration of apices, even though we have not demonstrated nerves synapsing with those SCCs by TEM (Kotrschal *et al.*, 1990).

Individual SCCs start with an apex carrying multiple, tiny sensory villi, reminiscent to the lamprey oligovillous cells (Whitear and Lane, 1983; Baatrup, 1984a,b). Multiple

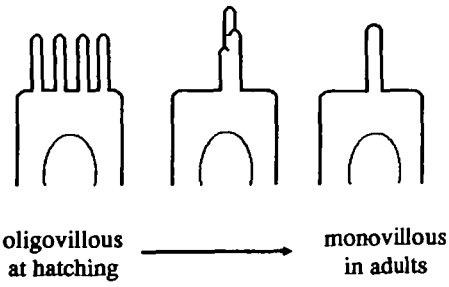

Figure 3 Diagram of changing shapes of the apices of solitary chemosensory cells in zebrafish from hatching to adulthood, from 'oligovillous' (left) to 'monovillous' (right), leaving the possibilities open that this change occurs via maturation of single cells or via a succession of cells.

apices have also been found in sturgeons, salmonids and other cyprinids, but seem less common in gadids and perches, where single, finger-like apices predominate (Kotrschal, 1991; Whitear, 1992; A. Hansen, unpublished data; K. Kotrschal and M. Whitear, unpublished data). Comparable cells in an elasmobranch (skate; Whitear and Moate 1994) terminated in a tuft of microvilli. It therefore seems that 'oligovillous' cells may represent the ancestral SCC condition and the 'monovillar' SCC morphology in adult teleosts may be considered as derived. Our present lack of knowledge of the sensory processes at the SCC apices, however, precludes speculations about the nature of the selection pressures which may have caused this shift from oligovillous towards monovillous apices.

In individual fish the monovillous SCC condition may be reached during growth either via a change of individual receptor cells apices or via a succession of cells, or both. This cannot be determined from the present data, especially as very little is known about SCC turnover rates. Experiments in adult rocklings revealed that SCCs may be more resilient after denervation than taste buds (Lane and Whitear, 1982; Whitear and Kotrschal, 1988). Therefore, an SCC turnover in the range of several weeks would not preclude either of the two hypotheses stated above and themonovillous condition could be reached either via maturation of individual cells or via a succession of SCCs (Figure 3).

Present results may fuel speculations on the evolution of the two taste systems, SCCs and taste buds (Kotrschal, 1996). Because the structural organization of SCC systems is less complex than that of taste buds (Kotrschal, 1991), it is plausible to assume that SCCs preceded taste buds in phylogeny and that buds were formed by aggregations of SCCs (Whitear, 1971). The opposite scenario, that SCCs derived from disaggregation of taste bud cells, is less plausible. The most relevant argument against this 'disaggregation hypothesis' is that oligovillous apices can only be found in SCCs, not in taste bud cells. It is unlikely that he ancestral apex condition would have been monovillous, then changed into oligovillous and finally changed back to the monovillous state in modern teleosts. The hypothesis that taste buds emerged from an aggregation of SCCs may be supported by the fact that SCCs appear earlier in ontogeny than taste buds. The more simple, and therefore more plausible, explanation would, however, be that this developmental pattern simply indicates direct functional demands (as discussed above) rather than evolutionary inertia: as exogenous feeding does not start before 5d AF, taste buds are not needed earlier. This does not explain why SCCs appear earlier than taste buds. If involved in predator avoidance, SCCs may be useful immediately after hatching.

The source of the considerable variation in SCC densities between developmental stages (Table 1) remains unclear. As preparation for SEM was standardized, it is unlikely that this variation reflects a methodological artifact. Possibly, SCC densities respond to environmental stimuli. In carp, for example, low pH may lead to enhanced SCC densities (Inger and Wendelaar Bonga, 1994). However, as the maintenance of the zebrafish prior to preparation was standardized, it is unlikely that environmental variability has caused this variability. It is also unclear whether the SCC densities reported are specific for zebrafish in general, or how much difference there is between different strains.

In conclusion, the early appearance of SCCs, prior to taste buds, and their steep increase in density (before 25d AF) from the larvae to early juveniles is compatible with the idea that SCCs mediate predator avoidance.

ACKNOWLEDGEMENTS

The authors gratefully acknowledge financial support by the Austrian FWF (Grants J-0367BIO, P-8412BIO and P-9602BIO) and by the German DFG (grant Ze 141/8-1). Special thanks to the Herzog von Cumberland-Stiftung and the Verein der Förderer der Konrad Lorenz Forschungsstelle for continuing support. The help of A. Menhart for TEM preparation and of

A. Zankl in processing the EM-prints is gratefully acknowledged. The manuscript benefited from comments by two anonymous reviewers.

REFERENCES

Baatrup, E. (1984a) Physiological studies on the pharyngeal terminal buds in the larval brook lamprey, *Lampetra planeri* (Bloch). *Chem. Sens.*, **10**, 549–558.

Baatrup, E. (1984b) *The Sense of Taste in Lamprey*. PhD thesis, University of Aarhus, with English summary.

Essler, H. and Kotrschal, K. (1994) High resolution analysis of swim path patterns of intact and olfaction-deprived minnows (*Phoxinus phoxinus*) stimulated with food and potential predator odour. *J. Fish Biol.*, **45**, 555–567.

Finger, T.E., Drake, S.K., Kotrschal, K., Womble, M. and Dockstader, K. (1991) Postlarval growth of the peripheral gustatory system in the Channel catfish, *Ictalurus punctatus*. *J. Comp. Neurol.*, **314**, 55–66.

Hansen, A. and Zeiske, E. (1993) Development of the olfactory organ of the zebrafish, *Brachydanio rerio*. *J. Comp. Neurol.*, **333**, 289–300.

Inger, Y. and Wendelaar Bonga, S.E. (1994) Cellular responses of the skin of carp (*Cyprinus carpio*) exposed to acidified water. *Cell Tissue Res.*, **275**, 481–492.

Kotrschal, K. (1991) Solitary chemosensory cells—taste, common chemical sense or what? *Rev. Fish Biol. Fish.*, **1**, 3–22.

Kotrschal, K. (1992) Quantitative scanning electron microscopy of solitary chemoreceptor cells in cyprinids and other teleosts. *Env. Biol. Fish.*, **35**, 273–282.

Kotrschal, K. (1995) Ecomorphology of solitary chemosensory cell systems in fish: a review. *Env. Biol. Fish.*, **44**, 143–155.

Kotrschal, K. (1996) Solitary chemosensory cells: why do primary aquatic vertebrates need another taste system? *Trends Ecol. Evolut.*, **11**, 110–114.

Kotrschal, K. and Essler, H. (1995) Goals and approaches in the analysis of locomotion in fish, with a focus on laboratory studies. *Rev. Fish. Sci.*, **3**, 171–200.

Kotrschal, K. and Finger, T. (1996) Secondary connections of the dorsal and ventral facial lobes in a teleost fish, the rockling (*Ciliata motella*). *J. Comp. Neurol.*, **370**, 415–426..

Kotrschal, K., Whitear, M. and Adam, H. (1984) Morphology and histology of the anterior dorsal fin of *Gaidropsarus mediterraneus* (Pisces, Teleostei), a specialized sensory organ. *Zoomorphology*, **104**, 365–372.

Kotrschal, K., Peters, R.C. and Atema, J. (1989) A novel chemosensory system in fish: do rocklings (*Ciliata mustela*, Gadidae) use their solitary chemoreceptor cells as fish detectors? *Biol. Bull.*, **177**, 328.

Kotrschal, K., Kinnamon, J. and Royer, S. (1990) High-voltage electron microscopy and 3-D reconstruction of solitary chemosensory cells and Di-I labeling of primary afferent nerves. In *Proceedings of the XII International Congress on Electron Microscopy*. San Fancisco Press, San Francisco, CA, pp. 412–413.

Kotrschal, K., Peters, R. and Atema, J. (1993a) Sampling and behavioral evidence for mucus detection in a unique chemosensory organ: the anterior dorsal fin in rocklings (*Ciliata mustela*: Gadidae: Teleostei). *Zool. Jahrb. Physiol.*, **97**, 47–67.

Kotrschal, K., Whitear, M. and Finger, T.E. (1993b) Spinal and facial innervation of the skin in the gadid fish *Ciliata mustela* (Teleostei). *J. Comp. Neurol.*, **331**, 407–417.

Kotrschal, K., Peters, R.C. and Doving, K.B. (1996) Chemosensory and tactile nerve responses from the anterior dorsal fin of a rockling, *Gaidropsarus vulgaris* (Gadidae, Teleostei). *Primary Sensory Neuron*, **1**, 297–308.

Lane, E.B. and Whitear, M. (1982) Sensory structures on the surface of fish skin. I. Putative chemoreceptors. *Zool. J. Linn. Soc.* **74**, 141–151.

Peters, R., Kotrschal, K. and Krautgartner, W.-D. (1991) Solitary chemoreceptor cells of *Ciliata mustela* (Gadidae, Teleostei) are tuned to mucoid stimuli. *Chem. Sens.*, **16**, 31–42.

Silver, W.L. and Finger, T.E. (1984) Electrophysiological examination of a non-olfactory, non-gustatory chemosense in the sea robin, *Prionotus carolinus*. *J. Comp. Physiol. A*, **154**, 167–174.

Wanzenböck, J., Zaunreiter, M., Wahl, C.M. and Noakes, D.L.G. (1996) Comparison of behavioural and morphological measures of visual resolution during ontogeny of roach (*Rutilus rutilus*) and yellow perch (*Perca flavescens*). *Can. J. Fish. Aquat. Sci.*, **53**, 1506–1512.

Westerfield, M. (ed., 1993). *The Zebrafish Book*. University of Oregon Press, Eugene, OR.

Whitear, M. (1965) Presumed sensory cells in fish epidermis. *Nature*, **208**, 703–704.

Whitear, M. (1971) Cell specialization and sensory function in fish epidermis. *J. Zool.*, **163**, 237–264.

Whitear, M. (1976) Identification of the epidermal 'Stiftchenzellen' of frog tadpoles by electron microscopy. *Cell Tissue Res.*, **175**, 391–402.

Whitear, M. (1992) Solitary chemoreceptor cells. In Hara, T.J. (ed.), *Chemoreception in Fishes*. Chapman & Hall, London, pp. 103–152.

Whitear, M. and Kotrschal, K. (1988) Chemosensory anterior dorsalfin in rocklings (*Gaidropsarus* and *Ciliata*, Teleostei, Gadidae): activity, fine structure and innervation. *J. Zool.*, **216**, 339–366.

Whitear, M. and Lane, E.B. (1983). Oligovillous cells of the epidermis: sensory elements of lamprey skin. *J. Zool.*, **199**, 359–384.

Whitear, M. and Moate, R.M. (1994). Chemosensory cells in the oral epithelium of *Raja clavata*. *J. Zool.*, **232**, 295–312.

Received on April 18, 1996; accepted on October 21, 1996