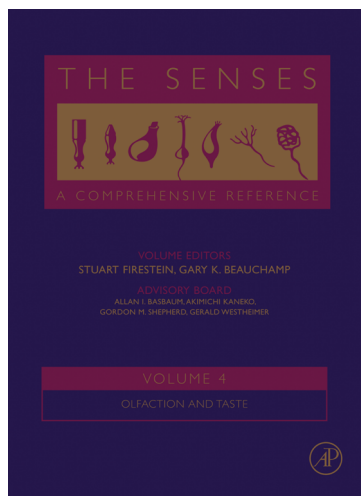


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4.04 Aquatic Animal Models in the Study of Chemoreception

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Glossary

accessory lobes (ALs) The paired deutocerebral neuropils in crustaceans that are connected to the olfactory lobes through interneurons but do not receive direct input from primary chemoreceptor neurons.

accessory olfactory bulb (AOB) The initial portion of the vertebrate central nervous system that processes information arriving from the vomeronasal organ.

aesthetasc sensilla Also called olfactory sensilla, they are the cuticular sensory structures of crustaceans that are located on the distal end of the lateral flagellum of the antennule and contain only olfactory receptor neurons that project only to the olfactory lobes.

antennal lobes The paired deutocerebral neuropils of insects that process odor information arriving from olfactory receptor neurons on the antennae; analogous to the vertebrate olfactory bulbs and the crustacean olfactory lobes.

antennules The first pair of antennae of crustaceans that are composed of basal segments at the proximal end and two flagella (lateral and medial) at the distal end.

chemoreceptor neuron (CRN) First-order neurons that detect environmental chemicals and transmit that information via their axonal projections into the central nervous system.

deutocerebrum The midbrain of arthropods (positioned between the protocerebrum and the tritocerebrum), which receives sensory information from the antenna; in crustaceans, the deutocerebrum includes the olfactory lobes, ALs, and lateral antennular neuropils.

facial lobe (FL) The primary gustatory nucleus of the medulla in teleosts that receives input from the facial taste nerve (cranial nerve VII) that innervates taste buds on the external body surface, lips, and rostral oral cavity.

lateral antennular neuropils (LANs) The paired deutocerebral neuropils of crustaceans that receive input from the CRNs and mechanoreceptor neurons that innervate the nonaesthetasc sensilla on the lateral and medial flagella of the antennules.

median antennular neuropil (MAN) The unpaired deutocerebral neuropil of crustaceans that receives input from the CRNs and mechanoreceptor neurons that innervate the nonaesthetasc sensilla of the antennules.

nonolfactory chemosensilla Also called non-aesthetasc chemosensilla, they are a diverse assortment of cuticular sensory structures located on the antennule of crustaceans, which project into the LANs and MAN.

olfactory bulb (OB) The initial portion of the central nervous system in vertebrates that processes odor information arriving from olfactory receptor neurons.

olfactory lobe (OL) In teleosts, the portion of the forebrain that receives input from the olfactory tract, the axons of mitral cells, the output neurons of the OB. In crustaceans, the deutocerebral neuropil that receives input from the axons of olfactory receptor neurons of the aesthetasc sensilla of the lateral flagellum of the antennule.

olfactory receptor (OR) Molecules, typically G-protein-coupled receptors, located on CRNs, to

which odorant molecules bind to initiate the olfactory transduction process.

olfactory receptor neuron (ORN) Primary receptor neurons whose dendrites contain the molecular ORs and whose axons project via the olfactory nerve into the olfactory region of the brain; in fish, the olfactory nerve is cranial nerve I projecting to the OB, and in crustaceans, it is a tract of the antennular nerve projecting to the OL.

perireception Events occurring around the olfactory and taste receptors that influence the chemical environment and the reception of chemical stimuli.

vagal lobe (VL) The primary gustatory nucleus of the medulla in teleosts that receives input from taste activity transmitted by the vagal taste nerve (cranial nerve X) that innervates taste buds within the oral cavity caudal to the first gill arch.

4.04.1 Introduction

The aim of this chapter is to provide a basic understanding of the chemosensory structures and mechanisms found in aquatic animals. Because of the vast literature on such an enormous topic, we have had to narrow considerably the scope of our review. First, we focus only on the major aquatic animal models in the study of chemoreception. We have selected two groups, representing the vertebrates and the invertebrates: the teleosts (bony fishes) and decapod crustaceans (spiny lobsters, clawed lobsters, crayfish, and crabs). Second, we focus on topics more aligned with biomedical than ecological issues: general structure of the chemosensory cells and their organization, and mechanisms of chemosensory transduction and the neural processing of chemical stimuli. Due to space limitations, topics such as pheromones (Stacey, N. E. and Sorensen, P. W., 2002; 2005) and the chemosensory basis of homing behavior (Døving, K. B., 1996; Døving, K. B. and Stabell, O. B., 2003) in fishes are not discussed. This chapter is also not an encyclopedic listing of how chemicals may influence various behaviors of aquatic organisms but focuses on chemosensory behaviors where there is better understanding of the neurophysiological substrate. Due to the necessary selection of research reports to include in this chapter from the numerous research articles that have allowed the field of aquatic chemoreception to progress to date, we take this opportunity to apologize to our coworkers whose work was not given the treatment that it deserves. Previously published excellent reviews in this field include Kleerekoper H. (1969), Hara T. J. (1982; 1992), Atema J. *et al.* (1989), Carr W. E. S. *et al.* (1990), Sandeman D. C. *et al.* (1992), Derby C. D. (2000), Weissburg M. J. (2000), Zimmer R. K. and Butman C. A. (2000), McClintock T. S. and Xu F. (2001), Ache B. W. (2002), Schachtner J. *et al.* (2005), Ache B. W. and

Young J. M. (2005), Moore P. A. and Berman D. A. (2005), and Atema J. and Steinbach M. A. (2007).

For terrestrial animals including mammals and insects, olfaction and taste are neatly distinguished based on the physical medium in which they operate. Olfaction is for volatile molecules that are delivered in air to the receptor epithelium, and taste is for water-soluble molecules that are delivered in an aqueous medium to the receptors. For fish and most crustaceans, which live in aquatic environments and whose chemosensory world is limited to water-soluble rather than to volatile chemical stimuli, this distinction between olfaction and taste is nonsensical.

Olfaction and taste are also distinguished within the vertebrates based on anatomical organization, regardless of the media in which the animals live. Olfaction is mediated by the olfactory nerve (cranial nerve (CN) I), and taste is mediated by modified epithelial (taste) cells innervated by CN VII (facial), IX (glossopharyngeal), or X (vagal), regardless of whether the animal is a human living in air, a fish living in water, or a frog that lives in both worlds. Yet this anatomical distinction, which works for vertebrates, is not instructive for the other ~97% of the animals – that is, the invertebrates.

A third way of distinguishing olfaction and taste is a functional one. Taste mediates more simple, reflexive behaviors – grabbing, biting, and swallowing (i.e., consummatory behavior), whereas olfaction mediates more complex behaviors, that is, search for chemicals from a distance, courtship behavior, and learning about odors (Atema, J. 1977; 1995); however, taste in some fish like catfishes mediates searching for distant food (i.e., appetitive behavior).

Another way to identify olfaction, at least for vertebrates and arthropods, is the organization of the first-order processing regions in the brains that receive the

peripheral chemosensory input. This region – the olfactory bulb (OB) of vertebrates, the antennal lobe of insects, and the olfactory lobe of crustaceans – is organized into glomeruli, which contain the synapses between the olfactory receptor neurons (ORNs) and second-order neurons. Furthermore, there is an odotopic organization to these glomeruli, in which odorants of different chemical categories generate distinctive activation patterns across the glomeruli.

In this chapter, olfaction and taste in fish is distinguished according to the vertebrate-specific pattern of nerve innervation, which is consistent with the olfaction-has-glomeruli model. For crustaceans, the glomerular definition of olfaction, and thus their olfactory pathway, is defined as the aesthetasc-olfactory lobe pathway (as described below). However, distinguishing between olfaction and taste creates a binary categorization that does not adequately describe the diversity of chemical senses of any animal, including fish and crustaceans. For example, this categorization applied to fish combines the oropharyngeal pathway and the extraoral pathway, even though they differ in cranial innervation (IX/X versus VII) and function – the former controlling reflexive swallowing (consummatory behavior) and the latter for locating distant food and taking it into the mouth (appetitive behavior). In addition, this categorization does not include the solitary chemoreceptor cells, which individually are anatomically similar to taste cells, but they are not organized into bud-like structures and they synapse with taste, trigeminal, or spinal nerves (Kapoor, B. G. and Finger, T. E., 2003). For crustaceans, this binary classification results in identifying all nonaesthetasc chemoreceptive neurons (CRNs) as taste, even though they are extremely diverse in how they are packaged into functional units, how they connect to the central nervous system (CNS), their organization within the CNS, and the behaviors they mediate (see below). Combining them into a single taste category is too simplistic and restrictive, and thus, in this chapter they are termed nonaesthetasc or nonolfactory chemosensors.

4.04.2 Olfactory Transduction

4.04.2.1 Introduction

ORNs of teleosts and crustaceans have served as important models not only for understanding the specifics of transduction in these species but also for elucidating fundamental and general principles of

olfactory transduction (Ache, B. W. and Zhainazarov, A. B., 1995; Bruch, R. C., 1996; Ache, B. W. 2002; Ache, B. W. and Young, J. M. 2005). Transduction processes that are well characterized and that we describe in this section include perireception (in crustaceans), molecular biology of receptor molecules (in fish), second messengers, ion channels, and kinases. Much has been learned about chemoreception in fishes from only a few animal models of the more than 25 000 extant teleost species. These model species include catfishes, zebrafish, goldfish, and salmonids. For crustaceans, the spiny lobster *Panulirus argus* and the clawed lobster *Homarus americanus* are the models of choice for studying olfactory transduction.

4.04.2.2 Fish

Genes for putative olfactory receptors (ORs) are identified from several teleosts (Ngai, J. *et al.*, 1993; Byrd, C. A. *et al.*, 1996; Cao, Y. *et al.*, 1998; Sun, H. *et al.*, 1999; Kondo, R. *et al.*, 2002). These ORs are G-protein-coupled receptors, although they are structurally highly dissimilar from the ORs of mammals and other vertebrates.

OR activation by odorants results in the formation of second messengers – cAMP and/or inositol 1,4,5-trisphosphate (IP₃)/diacylglycerol (DAG). These second messengers lead to the depolarization of ORNs via the gating of (1) cyclic nucleotide-gated (CNG) channels and subsequently Ca²⁺-activated Cl⁻ channels (for the cAMP pathway), which amplifies the odor-activated signal (Schild, D. and Restrepo, D., 1998), (2) ciliary IP₃ channels (Bruch, R. C., 1996; Schild, D. and Restrepo, D., 1998), or (3) TRPC2 (transient receptor potential) channels (Sato, Y. *et al.*, 2005), leading in all three cases to increasing intracellular Ca²⁺. Although excitatory conductances have only been described to date, ORNs of teleosts also exhibit odorant-induced suppression (Kang, J. and Caprio, J., 1995b), but little definitive information is known in fish about the responsible mechanisms. The cAMP pathway is the generally accepted excitatory mechanism for the ciliated ORNs (cORNs) of tetrapods, especially mammals (Gold, G. H., 1999), although recent evidence suggests that odor transduction in mammals likely also involves a cAMP-independent pathway (Lin, W. *et al.*, 2004). However, in microvillous receptor neurons of the vomeronasal system, a DAG transduction pathway was recently implicated (Lucas, P. *et al.*, 2003). Fish, however, do not possess a vomeronasal

system, and both cORNs and microvillous ORNs (mORNs) are present in the main olfactory epithelium. Recent evidence from teleosts suggests that specific odorants activate ORs and the cAMP second messenger system in cORNs (Sato, K. and Suzuki, N., 2000; Hansen, A. *et al.*, 2003; Schmachtenberg, O. and Bacigalupo, J., 2004; Sato, Y. *et al.*, 2005), whereas other odorants activate members of the V2R gene family and the IP₃ (and possibly the diacyl glycerol) pathway of mORNs (Specca, D. J. *et al.*, 1999; Hansen, A. *et al.*, 2003; Sato, Y. *et al.*, 2005).

A recent molecular modeling study identified key amino acid residues that are involved in ligand binding and selectivity for the L-arginine OR (a V2R olfactory receptor) in goldfish (Luu, P. *et al.*, 2004). Identified was a key residue, methionine 389, in goldfish OR 5.24 that provides OR selectivity to basic amino acids, and another residue, lysine 386, in zebrafish that is critical for OR selectivity to acidic amino acids. Although neither the odorant nor receptor types are known for crypt ORNs, the transduction mechanism in channel catfish likely utilizes G α_o (Hansen, A. *et al.*, 2003), whereas in goldfish the same crypt ORN expresses both G α_o and G α_q (Hansen, A. *et al.*, 2004).

4.04.2.3 Crustaceans

The importance of perireceptor events, which influence the odorant environment around the receptors, has been well studied in spiny lobsters. Flicking of the olfactory organ leads to periodic loading and unloading of chemical stimuli around the densely packed aesthetascs (Schmitt, B. C. and Ache, B. W., 1979; Koehl, M. A. R. *et al.*, 2001). Lobsters, fish, and other aquatic animals do not appear to have odorant-binding proteins, whose function in terrestrial animals may include delivery of odorants to receptors or even be more directly central to receptor activation (Vogt, R. G., 2005; Xu, P. *et al.*, 2005). Lobsters, however, do have several perireceptor elements that are involved in inactivating or removing odorants from the receptor environment. These include ectoenzymes, such as ectonucleotidases, to convert the excitatory compounds AMP and ATP into non-excitatory adenosine, and transporters for odorants such as taurine, adenosine, and glutamate (Carr, W. E. S. *et al.*, 1990).

Despite the fact that genes for ORs are not yet identified in any crustacean, other lines of inquiry, including biochemical, molecular, and physiological, reveal much about the nature of these receptors and

their role in transduction. Patch clamp (Hatt, H. and Ache, B. W., 1994), binding studies (Olson, K. S. *et al.*, 1992), and transmission electron microscopy (Blaustein, D. N. *et al.*, 1993) demonstrate odorant-binding receptors on the dendritic membrane of ORNs. Furthermore, biochemical analysis of receptor–ligand binding shows that high (nM to pM) and low (μ M) affinity sites for taurine, AMP, glutamate, and arginine receptors on the ORN dendrites (Olson, K. S. *et al.*, 1992; Michel, W. C. *et al.*, 1993; Olson, K. S. and Derby, C. D., 1995).

These receptors are G-protein-coupled receptors, as demonstrated by physiological, biochemical, and molecular studies. Odor-activated physiological responses of ORNs are affected in predictable ways by blockers or activators of G proteins (Fadool, D. A. *et al.*, 1995). In addition, several G-protein subunits (G α_i , G α_s , G α_q , and G β), phospholipase C- β (PLC- β), and a G-protein-coupled receptor kinase are identified from cDNA libraries of the antennules of lobsters (*Homarus*) (McClintock, T. S. and Xu, F., 2001). Furthermore, functional assays demonstrate a role for these molecules in either activation or desensitization of odorant responses (McClintock, T. S. and Xu, F., 2001).

Lobster ORNs have two distinct G-protein-coupled second messenger pathways (Ache, B. W., 2002). One of these is excitatory, mediated by phosphoinositol signaling. In this pathway, InsP₃ activates a cation-selective channel, leading to an inward conductance that normally depolarizes the cell. The other G-protein pathway is inhibitory, mediated by cyclic nucleotide signaling. In this pathway, cAMP activates an outward conductance, carried by K⁺ through a CNG K⁺ channel (Michel, W. C. and Ache, B. W., 1992), and suppresses a steady-state Cl⁻ channel (Doolin, R. E. *et al.*, 2001; Doolin, R. E. and Ache, B. W., 2005a). Both excitatory and inhibitory pathways are present in the same ORN, as revealed by electrophysiological recordings from both individual cells (McClintock, T. S. and Ache, B. W., 1989; Michel, W. C. *et al.*, 1991) and from patches of ORN dendritic membranes (Hatt, H. and Ache, B. W., 1994).

A given odorant can be excitatory to some ORNs and inhibitory to others (Michel, W. C. *et al.*, 1991) and can activate either the inward or the outward conductance (Doolin, R. E. and Ache, B. W., 2005b). Furthermore, it appears that the two inhibitory conductances, Cl⁻ and K⁺, are not odor specific (Doolin, R. E. and Ache, B. W., 2005b). Thus, no odorant is exclusively excitatory or inhibitory across cells, and

no receptor exclusively mediates excitation or inhibition.

These two second messenger-mediated pathways are the first step in odor activation, and this is followed by a second step that amplifies this signal and sets the gain of the ORN (Ache, B. W., 2002). This amplification involves a nonselective cation channel, which is a member of the TRP channel family (Zhainazarov, A. B. *et al.*, 2001; Bobkov, Y. V. and Ache, B. W., 2005) and may be functionally similar to the Ca^{2+} -activated Cl^- channel that participates in odor detection in mammals (Kleene, S. J., 1997).

These and other results lead to the conclusion that individual ORNs of spiny lobsters contain a rich diversity of transduction molecules. Together, these diverse intracellular pathways allow ORNs to do substantial processing and integration (Ache, B. W. *et al.*, 1998). In addition, ORN responsiveness can be modulated by gamma-aminobutyric acid (GABA_A) receptors on the soma (Zhainazarov, A. B., *et al.*, 1999) and by centrifugal synaptic inhibition at their axonal terminals (Wachowiak, M. *et al.*, 2002). An important question is, whether having ORNs with such complex integration properties is a feature of simpler animals (i.e., invertebrates), which have fewer ORNs than vertebrates, or whether vertebrate ORNs are similarly complex. Although this topic is hotly debated, recent evidence indicates that mammalian ORNs have both cAMP and IP_3 pathways that can interact in complex ways (Spehr, M. *et al.*, 2002; Zhainazarov, A. B. *et al.*, 2004).

4.04.2.4 Overview

Olfactory transduction in teleosts and crustaceans has many commonalities with each other and with other characterized ORNs. ORs are G-protein-coupled receptors linked via second messenger pathways to ion channels. A diversity of olfactory transduction cascades that lead to the activation of CNG, IP_3 , and/or TRPC2 channels exist. Other channels are secondarily activated, which in turn amplify the signal. Single ORNs of teleosts and lobsters can have more than one second messenger pathway. In lobsters, these include specific excitatory and inhibitory pathways. For teleosts, dual excitatory ORN pathways are identified, but it is currently unknown whether odorant-induced suppression requires an additional inhibitory transduction pathway, or whether specific odorants can affect the resident pathways to result in suppression (Hallem, E. A. *et al.*, 2004). Multiple transduction cascades in

individual cells can enhance the diversity of odor responses generated by ORNs. Perireceptor events are important to aquatic animals – for example, ectoenzymes and transporters clean up the odor environment around the ORs – but other perireceptor processes not relevant to aquatic animals are absent – for example, no odorant-binding proteins.

4.04.3 Properties of the Peripheral Chemical Detectors

4.04.3.1 Introduction

Peripheral chemosensory systems have the task of extracting biologically important information about the type, amount, and temporal dynamics of environmental chemicals and sending this information to the CNS. In this section, we review the organization of peripheral olfactory systems of fish and crustaceans and describe how they perform these important tasks.

4.04.3.2 Fish

4.04.3.2.1 Specificity of single olfactory receptor neurons

Although numerous studies in fishes tested amino acids as odorant stimuli, few investigations focused primarily on the odorant specificities of individual ORNs (MacLeod, N. K., 1976; Meredith, M. and Moulton, D. G., 1978; Meredith, M., 1981; Restrepo, D. *et al.*, 1990; Ivanova, T. T. and Caprio, J., 1993; Kang, J. and Caprio, J., 1995a; 1995b; 1997; Sato, K. and Suzuki, N., 2001; Friedrich, R. W. and Laurent, G., 2004). Electrophysiological studies in the channel catfish indicated that single ORNs had ongoing spontaneous activity ($\sim 5 \text{ spikes s}^{-1}$) that could be elevated or suppressed in response to amino acids (Kang, J. and Caprio, J., 1995b; 1997); furthermore, response suppression (25%) was the dominant response type (13% excited; 62% nonresponsive). A few single ORNs showed excitation to some amino acids and suppression to others. In an innovative study to reveal odor-induced spike activity of single ORNs whose responses were likely to have been shunted from an extracellular recording electrode due to the high ionic content of the pond water bathing the olfactory organ, the olfactory organ of bullhead catfish was bathed in water of low ionic content (Valentinčič, T. *et al.*, 2005). ORNs having low or no spontaneous activity were excited by specific amino acids. That excitation is the major type of

response to amino acids in teleost ORNs was also reported for zebrafish where only 4% (3 of 76 ORNs) were suppressed by an amino acid (Friedrich, R. W. and Laurent, G., 2004). None of these reports provided evidence for the existence of specific neuron types that were excited by particular amino acids.

Current unpublished data (Nikonov A. A. and Caprio J.), however, indicate the existence of ORNs that are highly specific to basic and neutral amino acids, respectively, similar to those reported for group I neurons in the OB (see section 4.04.4.2.4) and FB (see Nikonov, A. A. and Caprio, J., 2007).

4.04.3.2.2 Correlation between olfactory receptor neuron type, transduction process, and odorant sensitivity

Several morphological types of ORNs occur in the olfactory epithelium of teleosts: ciliated (c), microvillous (m), and crypt (cp) (Figure 1). However, controversy exists as to whether these different cell types process specific types of odorants (see Eisthen, H. L., 2004, for a review). A whole-cell patch clamp study of isolated ORNs from rainbow trout indicated, however, that mORNs responded specifically to amino acids, whereas cORNs were more broadly tuned, responding to a wide variety of odorants including amino acids (Sato, K. and Suzuki, N., 2001). That mORNs also respond to amino acids was indicated via activity-dependent labeling by the ion channel permeant probe, agmatine, in zebrafish (Lipschitz, D. L. and Michel, W. C., 2002). Additional evidence to suggest that mORNs respond to amino acids was obtained in the goldfish where a V2R receptor for L-arginine was shown to couple to PLC and IP₃ production and be expressed in ORNs whose cell bodies were located superficially in the olfactory epithelium, a characteristic of mORNs (Specia, D. J. *et al.*, 1999). Evidence that cORNs also respond to amino acids, as reported above for rainbow trout, comes from a perforated patch study of isolated cORNs in a marine fish, the Cabinza grunt (*Isacia conceptionis*). cORNs in this marine species responded to amino acids through the activation of CNG channels and calcium-activated conductances (Schmachtenberg, O. and Bacigalupo, J., 2004), confirming a similar report for cORNs in rainbow trout (Sato, K. and Suzuki, N., 2000).

The recent determination of the odotopic organization within the OB of a few species of teleosts also provided the pathway to link odorant type with receptor cell morphology. By placing DiI, a postmortem retrograde tracer, into select and discrete regions

of the teleost OB shown to be excited by specific classes of odorants, a direct link between ORN morphology and the type of odorant detected was recently derived from studies in crucian carp, channel catfish, and goldfish. For crucian carp, the evidence suggests that mORNs respond to food odors (Hamdani, E. H. *et al.*, 2001a), whereas cORNs are involved in the detection of alarm substances (Hamdani, E. H. and Døving, K. B., 2002). For channel catfish, the combination of a variety of experimental procedures (light and electron microscopy, *in situ* hybridization, immunocytochemistry, electrophysiology, and pharmacology) provided the necessary information to link the specific anatomical type of ORN with the type of molecular OR, its specificity for odorant type, and its probable mechanism of transduction (Hansen, A. *et al.*, 2003). In channel catfish, bile salt odorants (possible socially relevant cues in teleosts; Sorensen, P. W. and Caprio, J., 1998), are detected primarily by ORs expressed within cORNs and transduced via the G α_{olf} /cAMP cascade, whereas nucleotides are detected by V2Rs expressed in mORNs but transduced via an unknown (neither G α_{olf} /cAMP nor G $\alpha_{q/11}$ /PLC) cascade; however, recent evidence in zebrafish suggests that nucleotides possibly activate TRPC2 channels expressed in mORNs (Sato, Y. *et al.*, 2005). Amino acid odorants in channel catfish activate both cORNs (expressing ORs) and mORNs (expressing V2Rs) but via different transduction pathways in the two types of ORNs (Hansen, A. *et al.*, 2003). For goldfish, cORNs also express ORs and G α_{olf} , and mORNs express V2R genes but are primarily immunoreactive for G α_o ; however, G α_{i-3} and G α_q are also indicated for mORNs that are shorter and that possess more stiff microvilli than other mORNs (Hansen, A. *et al.*, 2004). Independent pharmacological evidence in goldfish also indicated that bile salt odorants and some amino acids are detected via the cAMP pathway and that amino acids also utilize the IP₃ transduction pathway (Rolen, S. H. *et al.*, 2003), which is consistent with previous molecular investigations (Bruch, R. C., 1996).

Sex pheromones in teleosts are gonadal steroids and prostoglandins (Sorensen, P. W. and Caprio, J., 1998). Recent evidence in crucian carp indicate that axons of cp neurons project to the ventral OB where they synapse with mitral cells whose axons form the lateral bundle of the medial olfactory tract and mediate sexual behavior (Hamdani, E. H. and Døving, K. B., 2006; Lastein, S. *et al.*, 2006; Weltzien, F. *et al.*, 2003).

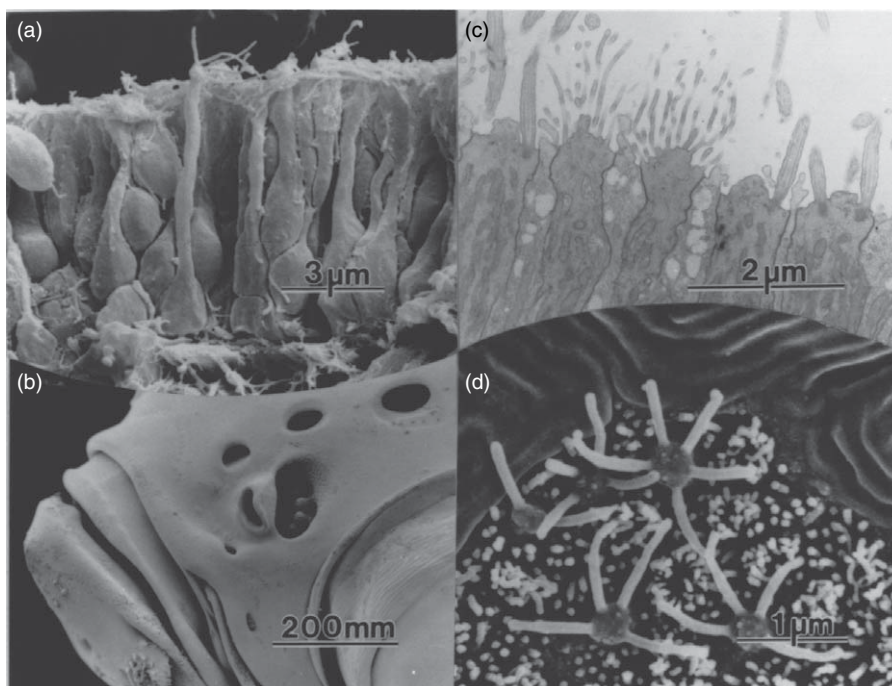


Figure 1 Telemost olfactory epithelia. (a) Scanning electron micrograph (SEM) of the adult sensory epithelium of a zebrafish, *Danio rerio*, showing some receptor cells with long dendrites, and some round receptor cells with short dendrites. (b) SEM of the olfactory organ of a zebrafish showing the organ, lateral line canals, and eye. (c) Transmission electron micrograph (TEM) of an apical longitudinal section of the olfactory epithelium of a goldfish, *Carassius auratus*, showing microvillous and ciliated olfactory receptor cells separated by supporting cells with vesicles. (d) SEM of the Indo-Pacific catfish, *Plotosus lineatus*, showing apical knobs of ciliated and microvillous receptor neurons. Reprinted from Sorensen, P. W. and Caprio, J. 1998. Chemoreception. In: *The Physiology of Fishes* (ed. D.H. Evans), 375–405. CRC Press LLC, by courtesy of Taylor and Francis Group, LLC. Courtesy: Eckart Zeiske and Anne Hanson.

4.04.3.3 Crustaceans

4.04.3.3.1 Organization

Being arthropods, crustaceans have an exoskeletal covering. Consequently, their chemosensory neurons are packaged into thin extensions of the cuticle, called setae or sensilla. A rich diversity of sensilla exists, which can be classified into olfactory and nonolfactory chemosensilla.

4.04.3.3.1.(i) Olfactory sensilla The olfactory sensilla, called aesthetascs, have been recognized in many crustacean taxa (Grünert, U. and Ache, B. W., 1988; Hallberg, E. *et al.*, 1992). Aesthetascs are distinguished by their peripheral structure and central connections. They are the only sensilla known to be innervated solely by chemosensory neurons and are typically richly innervated (hundreds of ORNs in some species such as spiny lobsters) (Derby, C. D. *et al.*, 2003). Aesthetascs are located on the paired first antennae (antennules) but only on the distal end of the lateral flagellum of the antennule (Figure 2). Chemical stimuli

pass through the porous cuticle of the aesthetascs and bind to receptor sites on the ORN dendrites. Each mature aesthetasc contains representatives of each of the functional types of ORNs, and thus, aesthetascs appear to be functional units of olfaction (Steullet, P. *et al.*, 2000b). The axons of the aesthetasc ORNs project ipsilaterally to the olfactory lobes (Schmidt, M. and Ache, B. W., 1996b) (see Section 4.04.4). Although aesthetascs are present in many crustacean taxa, they differ in their structure in functionally significant ways. For example, the organization of aesthetascs differs in aquatic, semiterrestrial, and terrestrial species (Ghiradella, H. *et al.*, 1968; Stensmyr, M. C. *et al.*, 2005). Aesthetasc sensilla contain not only sensory neurons but also supporting cells, which have functional overlap with supporting cells in insect and vertebrate olfactory organs. For example, supporting cells include (1) secretory cells associated with glands that produce a substance that likely coats the external surface of the aesthetasc cuticle (Schmidt, M. and Derby, C. D., 2005); (2) cells that secrete the sensillar cuticle; and (3) cells that produce ectoenzymes and

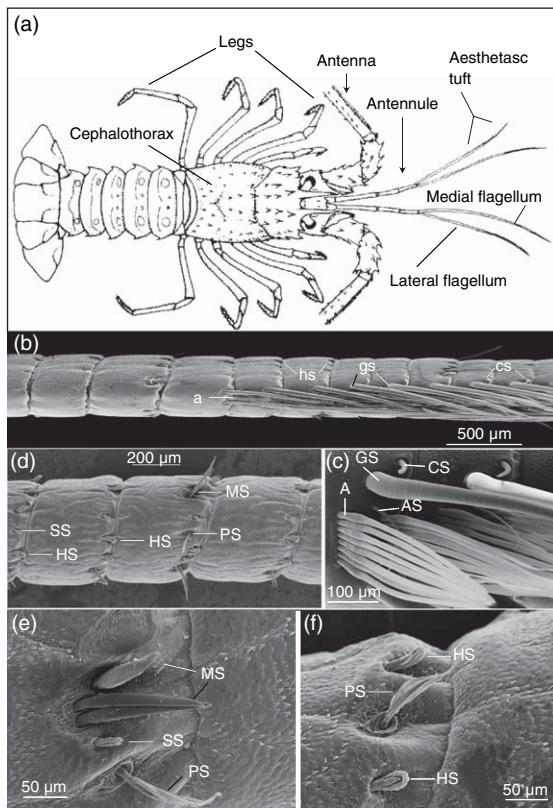


Figure 2 Peripheral chemosensory structures in spiny lobsters. (a) Drawing of a spiny lobster, *Panulirus argus*, showing the chemosensory organs. Each of the antennules (first antennae) has a medial flagellum and lateral flagellum. The sensory neurons are packaged into sensilla. The aesthetasc tuft, which contains aesthetasc sensilla and other associated sensilla (shown in the right half of b and in c), is located on the ventral side of the distal half of the lateral flagellum. Chemoreceptor neurons that innervate sensilla other than aesthetascs are called nonaesthetasc or nonfactory chemoreceptor neurons. (b–f) Scanning electron micrographs of antennular sensilla. (b) The lateral flagellum at the boundary of the aesthetasc tuft. The aesthetasc tuft is to the right in this figure. The flagellum is divided into annuli, 12 of which are shown here. Each annulus in the aesthetasc region bears a similar complement of sensilla (shown in c), and each annulus in the nonaesthetasc region bears a similar complement of setae (shown in d–f). (c) Tuft region of the lateral flagellum. (d) Nontuft region of the lateral flagellum; the medial flagellum has similar types and organization of sensilla to this region. (e, f) High magnification views of nonaesthetasc regions of the lateral flagellum, showing four types of nonaesthetasc sensilla. A, aesthetasc sensillum; AS, asymmetric sensillum; CS, companion sensillum; GS, guard sensillum; HS, hooded sensillum; MS, medium-length simple sensillum; PS, plumose sensillum; SS, setuled sensillum. From figure 1 of Steullet, P., Dudar, O., Flavus, T., Zhou, M., and Derby, C. D. 2001. Selective ablation of antennular sensilla on the Caribbean spiny lobster *Panulirus argus* suggests that dual antennular chemosensory pathways mediate odorant activation of searching and localization of food. *J. Exp. Biol.* 204, 4259–4269, used with permission.

transporters that regulate the chemical environment of the fluid bathing the sensory cells (Carr, W. E. S. *et al.*, 1990; Gleeson, R. A. *et al.*, 1992).

4.04.3.3.1(ii) Nonolfactory chemosensilla Nonolfactory sensilla are on all appendages and most of the body surface. Some of these, such as those on the mouthparts, are clearly gustatory in function, in that they control the ingestion of food (Derby, C. D. and Atema, J., 1982; Garm, A. *et al.*, 2005). Others are found on the legs, antennules, antennae, and general body surface (Derby, C. D., 1982; Schmidt, M. and Gnatzy, W., 1984; Cate, H. S. and Derby, C. D., 2001; 2002; Schmidt, M. and Derby, C. D., 2005), and consequently, these have many different functions. The nonolfactory chemosensilla are morphologically diverse, but they do have several common features. They are at least bimodally innervated, containing not only chemosensory neurons but also mechanosensory neurons (Schmidt, M. and Gnatzy, W., 1984; Cate, H. S. and Derby, C. D., 2002; Garm, A. *et al.*, 2005). Nonolfactory sensilla are more sparsely innervated than aesthetascs, typically with two or three mechanoreceptor neurons and fewer than 20 CRNs. Their cuticle is thick and not porous, and thus, chemicals enter through a terminal pore. The chemosensory neurons of these sensilla do not project to the olfactory lobe but rather to other neuropils in the CNS. This occurs even for nonolfactory antennular chemosensory neurons, which project ipsilaterally to the lateral antennular neuropils (LANs) and to the median antennular neuropil (MAN) (Schmidt, M. and Ache, B. W., 1996a) (see Section 4.04.4).

4.04.3.3.2 Sensitivity

4.04.3.3.2.(i) Response thresholds Olfactory and nonolfactory receptor neurons often differ in sensitivity. For *P. argus*, the sensitivity of antennular CRNs is much greater than for mouthpart CRNs (Thompson, H. A. and Ache, B. W., 1980; Derby, C. D. *et al.*, 1991; Garm, A. *et al.*, 2005). For example, thresholds for antennular CRNs (which include aesthetasc ORNs) are typically nanomolar and sometimes lower, whereas mouthpart CRNs have micromolar thresholds.

4.04.3.3.2.(ii) Tuning Crustaceans are sensitive to chemicals associated with most biologically important items, including food stimuli, social and sexual pheromones, settlement factors, and defensive compounds (Atema, J., 1988; Carr, W. E. S., 1988; Zimmer, R. K. and Butman, C. A., 2000; Kicklighter, C. E. *et al.*, 2005). Differences in sensitivity among species are partly

attributable to the habitat in which they live. For example, for aquatic crustaceans, all sensors, including olfactory, are stimulated by water-soluble compounds. For terrestrial crustaceans, the antennules are sensitive to volatile chemicals, whereas the legs and mouthparts are sensitive to water-soluble stimuli (Rittschof, D. and Sutherland, J. P., 1986; Wellins, C. A. *et al.*, 1989; Stensmyr, M. C. *et al.*, 2005). Trophic level also accounts for interspecific differences in sensitivity. For example, carnivores such as *Panulirus* and *Homarus* have CRNs that respond to small, nitrogen-containing compounds—amino acids, amines, nucleotides, and peptides—that are prevalent in tissues of their animal prey and are relatively insensitive to carbohydrates and sugars (Zimmer-Faust, R. K. and Case, J. F., 1982a; Carr, W. E. S., 1988; Derby, C. D. and Atema, J., 1988; Zimmer-Faust, R. K., 1993). Herbivores and omnivores, such as fiddler crabs (Weissburg, M. J. and Zimmer-Faust, R. K., 1991), ghost crabs (Robertson, J. R. *et al.*, 1981; Trott, T. J. and Robertson, J. R., 1984), kelp crabs (Zimmer-Faust, R. K. and Case, J. F., 1982b), and crayfish (Ashby, E. A. and Larimer, J. L., 1965), often are sensitive to sugars common to plants, bacteria, and diatoms, as well as to some amino acids.

4.04.3.3.2.(iii) Specificity The CRNs of crustaceans tend to be narrowly tuned, responding with high specificity to one compound or a set of structurally related compounds. Examples include CRNs of *H. americanus* tuned to predominantly to taurine, hydroxy-L-proline, or L-glutamate (Voigt, R. and Atema, J., 1992), and antennular CRNs of *P. argus* tuned primarily to taurine, glutamate, AMP, ATP, or ammonium (Derby, C. D. *et al.*, 1991; Derby, C. D., 2000). In the crayfish *Austropotamobius torrentium*, leg CRNs are less narrowly tuned but still have specificity to one of the following classes of compounds—amino acids, pyrimidines, or amines (Bauer, U. and Hatt, H., 1980; Bauer, U. *et al.*, 1981).

Within a species, there may be differences in specificity of CRNs in different organs. For example, in *P. argus*, individual antennular CRNs have narrower response profiles than mouthpart chemoreceptors (Garm, A. *et al.*, 2005).

Physiological studies of CRNs suggest that there are as many as a dozen types of molecular receptors that are heterogeneously distributed across the CRNs. Do single cells express a single receptor type, or more than one? It is often stated that ORNs of mammals and *Drosophila* express only one type of receptor, but this idea is being questioned in the face of new data (Mombaerts, P., 2004; Goldman, A. L.

et al., 2005), and it certainly is not true for CRNs in other animals such as *C. elegans* (Troemel, E. R., 1999). Although genes for chemoreceptors have not been identified in any crustacean, physiological studies strongly suggest that a given cell type has multiple receptor types, each coupled to either an excitatory or an inhibitory transduction cascade (see Section 4.04.2). However, the density of the receptor types on a CRN may vary, thus creating high response specificity (Cromarty, S. and Derby, C., 1997).

4.04.3.3.3 Temporal properties

Chemical stimuli in nature, even those released at a constant rate from a single odor source, are patchy and discontinuous. This is due to turbulence, which is ever-present though at different degrees in different environments. The temporal features of chemical stimuli influence the response pattern of a cell and consequently an animal's ability to find an odor source (Atema, J., 1995; Weissburg, M. J., 2000; Zimmer, R. K. and Butman, C. A., 2000; Koehl, M. A. R. *et al.*, 2001). Since the ability of CRNs to resolve temporal fluctuations determines the animal's ability to respond to them, it is important to identify these properties. The frequency following of CRNs is dependent on adaptation and disadaptation rates (Atema, J., 1995). Many crustacean CRNs have phasotonic response profiles, although this varies across cells (Borroni, P. F. and Atema, J., 1988; 1989; Weissburg, M. J. and Derby, C. D., 1995; Garm, A. *et al.*, 2005). CRNs on *Homarus* antennules can follow 4–5 odor pulses per second (Gomez, G. and Atema, J., 1996a; 1996b; Gomez, G. *et al.*, 1999). Since temporal variability of this magnitude is common in aquatic environments (see the above references), this ability to follow such pulses is obviously adaptive.

4.04.3.4 Processing Chemical Information by Single Peripheral Chemoreceptor Neurons of Crustaceans

To summarize our knowledge of neural coding by crustacean peripheral CRNs using a parallel construction with the section [Processing Taste Information by Single Peripheral Taste Fibers](#) by single peripheral taste fibers, we offer the following points:

1. Information about different classes of chemical stimuli can be transmitted to the CNS by different groups or types of CRNs; for example, the antennules, legs, and mouthparts of spiny lobsters and American lobsters have some CRNs most sensitive

to one or several amino acids, others most sensitive to one or several adenine nucleotides, and others most sensitive to ammonium ions (Voigt, R. and Atema, J., 1992; Derby, C. D., 2000; Garm, A. *et al.*, 2005).

2. The specificity of single CRNs is not limited to a particular class of chemical stimuli, although the strongest stimulus is usually several orders of magnitude more stimulatory than other stimuli; for example, taurine-best cells on antennules of spiny lobsters may also be sensitive to other amino acids, AMP, and ammonium, although the thresholds for taurine are 100–10 000 lower than for the other stimuli (Cromarty, S. and Derby, C., 1997).
3. Different CRNs having widely different tuning to members of the same class of chemicals can exist within the sensory appendage; for example, populations of CRNs with different amino acid specificities occur on the antennules, legs, and mouthparts of lobsters (Derby, C. D. *et al.*, 1991; Voigt, R. and Atema, J., 1992; Derby, C. D., 2000).
4. CRNs in different sensory appendages within the same species can have different chemical specificities; for example, in the American lobster, CRNs highly sensitive to hydroxy-L-proline are more abundant on the antennule than the legs or mouthparts (Voigt, R. and Atema, J., 1992).
5. The proportion and types of CRNs in different sensory appendages can vary within the same species. For example, cells with best responses to either hydroxy-L-proline, taurine, L-arginine, L-glutamate, betaine, and ammonium chloride occur in the antennules, legs, and mouthparts of the American lobster but in different proportions (Voigt, R. and Atema, J., 1992).
6. L-Amino acids are much more stimulatory than their respective D-isomers, but receptors for some D-isomers do exist (Michel, W. C. *et al.*, 1993).
7. Stimulus quantity appears to be coded by the frequency of action potentials generated. For individual CRNs, some have a dynamic concentration–response range of 5–6 log units of stimulus concentration, while others have more truncated concentration–response relationships. The absolute concentrations to which cells are responsive can differ widely (Derby, C. D. and Atema, J., 1988; Borroni, P. F. and Atema, J., 1988; 1989; Derby, C. D. *et al.*, 1991; Cromarty, S. and Derby, C., 1997).
8. Except for one report (Hatt, H., 1986), CRNs are not known to be sensitive to mechanical stimulation. However, they can respond to temperature and salinity changes (Schmidt, M., 1989).

4.04.3.5 Overview

Fish and crustaceans have a diversity of peripheral chemoreceptors. In teleosts, three types of ORNs have been identified: ciliated, microvillous, and crypt, which differ in their mode of transduction. The crustacean olfactory organ has many types of chemosensory neurons packaged into units called sensilla, but one of these sensillar types – the aesthetascs – is an olfactory sensillum based on its organization and central projections. The ability of fish and crustacean ORNs to detect and identify the type, amount, and temporal dynamics of chemical stimuli is well understood. ORNs generally have low spontaneous spiking activity, which is more usually increased but sometimes decreased by chemical stimulation. The population of ORNs on an olfactory organ responds to many chemicals, with small nitrogenous compounds, such as amino acids, amines, and nucleotides being the most effective. However, individual ORNs differ in their response specificities, which can be quite narrow. The response of ORNs increases in a concentration-dependent manner. Crustacean ORNs can follow pulses of chemicals up to at least 4–5 per second.

4.04.4 Organization of Central Olfactory Pathways

4.04.4.1 Introduction

The organization of the olfactory pathway of fishes and crustaceans has many elements common with that of other vertebrates, arthropods, and even other taxa. This section examines the connectivity between the olfactory periphery and CNS, the organization of the olfactory CNS, and how odor responses from the periphery are shaped by synaptic interactions within the olfactory CNS.

4.04.4.2 Fish

4.04.4.2.1 Organization of the olfactory bulb

Although the general anatomical organization of the olfactory system is similar across the vertebrates, the structure of the neural connections within the OB can be different between fish and mammals (reviewed in Dryer, L. and Graziadei, P. P. C., 1994). In contrast to mammals where a single mitral cell generally projects a single primary dendrite to a single large (50–200 μm in diameter), well-defined

glomerulus, mitral cells in the fish OB can project primary dendrites to several different, small (10–20 µm in diameter), and less well-defined glomeruli (Mori, K., 1995). However, the organization of the teleost OB may not be that radically different than in some mammals, since ~20% of the mitral cells in the main OB in rabbits possess multiple primary dendrites (Mori, K. *et al.*, 1983).

The anatomical organization in the teleost OB is similar to that observed in the accessory olfactory bulb (AOB) of the vomeronasal system in tetrapods where AOB mitral cells also have multiple apical dendrites, each of which innervates a small glomerulus (Satou, M., 1990; Takami, S. and Graziadei, P. P. C., 1991). This organization suggests that mitral cells in both the fish OB and the tetrapod AOB integrate odorant information from different types of ORNs (i.e., those expressing different molecular receptors) that provide input to the different glomeruli. A recent tracing study in the mouse, however, indicated that AOB mitral cells innervate glomeruli that only receive input from the same receptor type (i.e., a homotypic connectivity) (Del Punta, K. *et al.*, 2002). It is currently unknown whether such a homotypic connectivity is characteristic of the teleost OB (Satou, M. *et al.*, 1983; Riddle, D. R. and Oakley, B., 1992). Depending on the particular species of teleost, the anatomical organization within the OB can vary. For example, a recent investigation of the zebrafish OB indicated that the majority (~80%) of the mitral cells observed possessed a single primary apical dendrite (Fuller, C. L. *et al.*, 2005).

The dendritic field of a single mitral cell in fish can extend for more than 300–400 µm from the soma (Kosaka, T. and Hama, K., 1982b; Oka, Y., 1983) and terminate either in discrete tufts or in diffuse, brush-like ending (Kosaka, T. and Hama, K., 1982b; Riddle, D. R. and Oakley, B., 1992). However, in spite of this extensive dendritic field, the neuronal activities on one side of the OB are not influenced much by those in the opposite side (Satou, M., 1990), suggesting that there is little crossing of dendritic fields to opposite sides of the bulb. The recent evidence for odotopy within the OB of channel catfish (Nikonov, A. A. and Caprio, J., 2001) supports the functional isolation between right and left OB. In other aspects, mitral cells within fish lack basal dendrites, and thus, lateral interactions between mitral cells occur at the bases of their primary dendrites (Ichikawa, M., 1976; Kosaka, T. and Hama, K., 1982a; Oka, Y., 1983). Furthermore, axons of fish mitral cells arise not only from cell bodies but from thick dendrites (Dryer, L. and Graziadei, P. P. C., 1994). Ruffed neurons, a cell type not observed in

tetrapods, are also located within the mitral cell layer in teleosts. These cells synapse with granule cells but not with ORNs (Kosaka, T. and Hama, K., 1979; 1981). Tufted and periglomerular cells are apparently lacking in the teleost OB (Satou, M., 1990; Dryer, L. and Graziadei, P. P. C., 1994).

4.04.4.2.2 Extrabulbar pathway

Although the vast majority of axons of ORNs project to the OB, in some teleosts, a small subset ORN axons pass through the OB and project directly to the ventromedial telencephalon (Bazer, G. T. *et al.*, 1987; Honkanen, T. and Ekstrom, P., 1990; Szabo, T. *et al.*, 1991; Riddle, D. R. and Oakley, B., 1992; Becerra, M. *et al.*, 1994; Hofmann, M. H. and Meyer, D. L., 1995). Little functional information is known about this extrabulbar primary olfactory pathway (Fujita, I. *et al.*, 1991), but it is distinct from that of the terminal nerve (CN 0) whose axons also pass through the OB and connect to specific forebrain (FB) nuclei (Demski, L. S. and Northcutt, R. G., 1983).

4.04.4.2.3 Odotopic representation of odorants in the teleost olfactory bulb

Indication that the teleost OB is divided into different zones, each processing a specific class of biologically relevant odor, was initially indicated by Thommesen G. (1978), based on the recording of local field potentials in salmonids. This study showed that the lateral OB was highly responsive to amino acids, whereas the medial OB was more selective to bile acids/salts. Other evidence came from anatomical studies also in a salmonid (rainbow trout) that olfactory information arriving at the OB from the olfactory epithelium was likely sorted chemotopically (Riddle, D. R. *et al.*, 1993) and not topographically (Riddle, D. R. and Oakley, B., 1991). More direct evidence for the existence of an odotopic map in the teleost OB was derived from additional investigations of field potential in salmonids (Døving, K. B. *et al.*, 1980; Hara, T. J. and Zhang, C., 1996), calcium (Friedrich, R. W. and Korsching, S. I., 1997; Fuss, S. H. and Korsching, S. I., 2001) and voltage (Friedrich, R. W. and Korsching, S. I., 1998) imaging of olfactory nerve terminals within the OB of zebrafish, and a single-unit study in the OB of the channel catfish (Nikonov, A. A. and Caprio, J., 2001). The exceptional optical imaging study of odor responses to amino acids of olfactory receptor terminals within glomeruli of the lateral OB in zebrafish (Friedrich, R. W. and Korsching, S. I., 1997) provided the direct evidence that both odor identity and concentration

are encoded by combinatorial glomerular activity patterns (Buck, L. B., 1996). Although some variations exist across the species of teleosts investigated, the general results of these studies indicate that food-related odors (i.e., amino acids and nucleotides) are mapped in OB regions (generally in the lateral OB) distinct from those of more socially relevant odors (bile salts, bile acids, and pheromones), which are mapped generally in the medial OB (Sorensen, P. W. *et al.*, 1991; Nikonov, A. A. and Caprio, J., 2001).

The medial–lateral distinction in odotopy within the teleost OB is consistent with mitral cell axons of the medial and lateral OB, respectively, projecting into the medial and lateral olfactory tracts (OTs) (Sheldon, R. E., 1912; Satou, M., 1990). The neuronal activities on one side of the fish OB are not influenced much by those on the opposite side, which is explained by limited dendritic fields of neurons in each part of the OB (Satou, M., 1990). In this respect, electrical stimulation experiments of distinct bundles of the OT in salmonids (Døving, K. B. *et al.*, 1980) and goldfish (Stacey, N. E. and Kyle, A. L., 1983; Demski, L. S. and Dulka, J. G., 1984) and behavioral investigations in crucian carp that had selected transections of different OT bundles (Hamdani, E. H. *et al.*, 2000; 2001a; 2001b; Weltzien, F. *et al.*, 2003) also support the previous evidence for a functional (food-related versus socially related) division between the medial and the lateral OB. This functional separation between types of odorants processed in medial and lateral OB is in sharp contrast to the two mirror-symmetric glomerular maps that exist in rodents (Lodovichi, C. *et al.*, 2003).

4.04.4.2.4 Odorant specificity of single olfactory bulb neurons

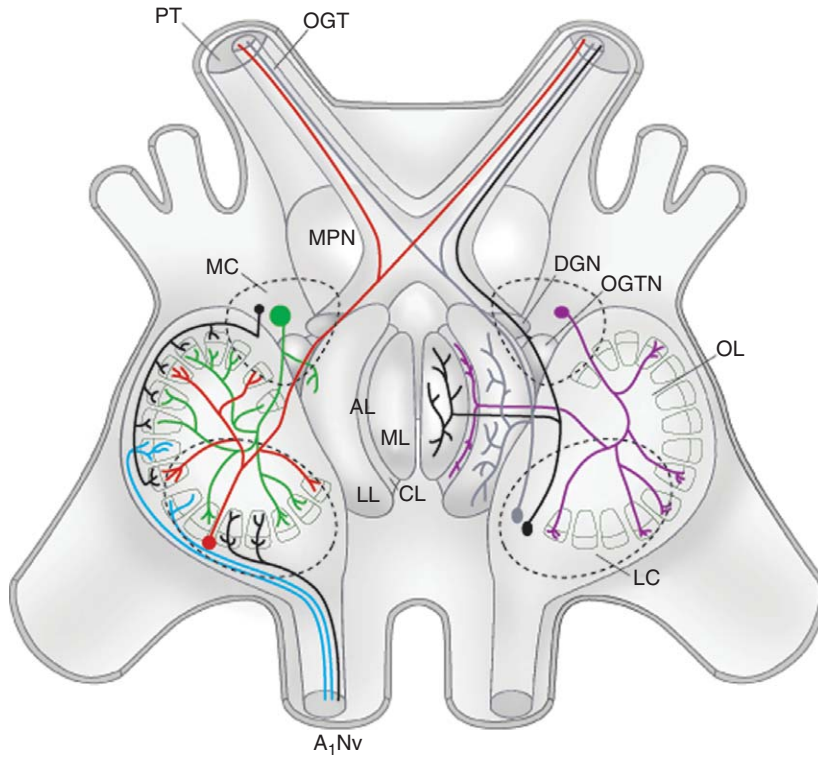
Electrophysiological investigations within the OB of the channel catfish (Nikonov, A. A. and Caprio, J., 2004) and zebrafish (Friedrich, R. W. and Laurent, G., 2004) provided information concerning the excitatory response spectrum to amino acids of single neurons (likely mitral cells) at the first synaptic level in the teleost olfactory system. Since the axons of ORNs expressing like ORs converge in the OB onto specific target glomeruli (Ressler, K. J. *et al.*, 1994; Vassar, R. *et al.*, 1994; Mombaerts, P. *et al.*, 1996; Strotmann, J. *et al.*, 2000), it is likely even in fish that the mitral cells whose apical dendrites innervate the respective glomeruli are tuned to odorants similarly as the ORNs that provide the input, but modified by intraglomerular circuitry (Yokoi, M. *et al.*, 1995; Sachse, S. and Galizia, G., 2002).

Two groups of neurons were recently identified in the OB of the channel catfish that were excited by

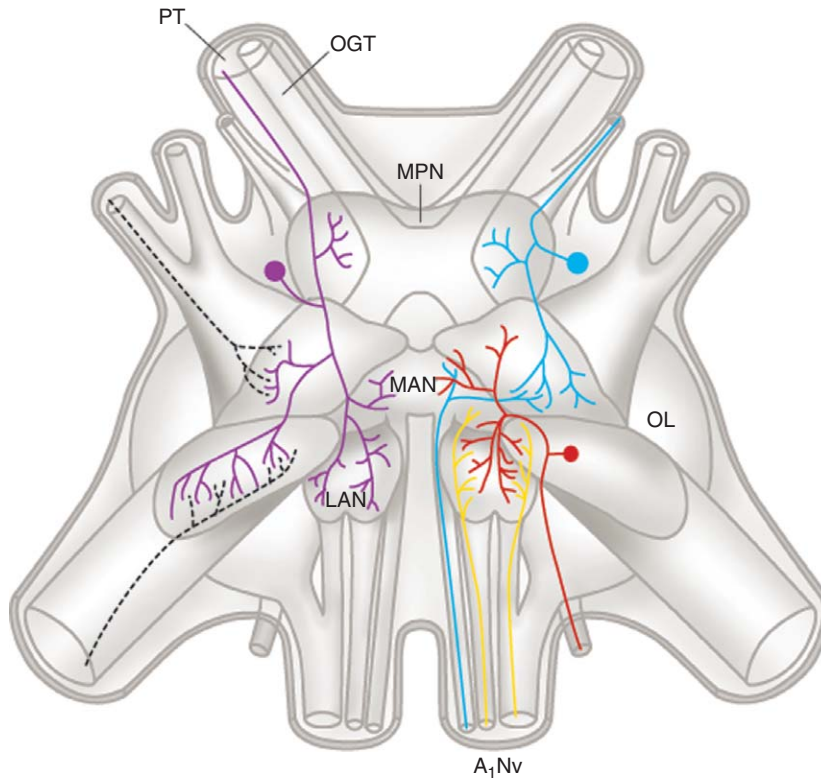
amino acids. Group I OB neurons ($n = 91$ (37%)) were highly specific to the type of amino acid, that is, excited by acidic, basic, or neutral (those possessing short and long side chains, respectively) amino acids, and group II OB neurons ($n = 154$ (63%)) were also excited by a second amino acid but only at ≥ 10 times higher odorant concentration (Nikonov, A. A. and Caprio, J., 2004). Tested with an expanded series of amino acids, a subset of the group I OB units that were selective for neutral amino acids also distinguished neutral amino acids possessing long linear side chains from those with branched side chains. With increasing stimulus concentration, however, responses broadened such that single OB neurons responded selectively to neutral amino acids having either linear or branched side chains, but not to amino acids with either acidic or basic side chains. These results are consistent with the identification of relatively independent molecular ORs for acidic, basic, and neutral amino acids that were previously indicated in teleosts from electrophysiological cross-adaptation (Caprio, J. and Byrd, R. P., Jr. 1984; Caprio, J., *et al.* 1989; Sveinsson, T. and Hara, T. J., 1990; Michel, W. C. and Derbidge, D. S., 1997), biochemical binding (Cagan, R. H. and Zeiger, W. N., 1978; Brown, S. B. and Hara, T. J., 1981; Rhein, L. D. and Cagan, R. H., 1983; Rehnberg, B. G. and Schreck, C. B. 1986; Lo, Y. H. *et al.*, 1991), and calcium imaging (Friedrich, R. W. and Korsching, S. I., 1997; Fuss, S. H. and Korsching, S. I., 2001) studies. Relatively independent ORs for neutral amino acids with short and long side chains, respectively, were also reported (Caprio, J. and Byrd, R. P., Jr. 1984; Bruch, R. C. and Rulli, R. D., 1988; Sveinsson, T. and Hara, T. J., 1990).

It is rather intriguing that recent electrophysiological data obtained in zebrafish also provide evidence for OB unit selectivity to the type of amino acid prior to a reported declustering of odor representations that occurred over the first 800 ms of the response (Friedrich, R. W. and Laurent, G., 2001; Laurent, G. *et al.*, 2001). However, a similar effect was not observed in OB unit responses in channel catfish analyzed over a 3 s response period (Nikonov, A. A. and Caprio, J., 2004). Of 78 group I units analyzed from the OB of the channel catfish (i.e., those that were originally determined to be selectively responsive to only Met, Ala, Arg, or Glu over 3 s of response), 81% and 85% were similarly classified when analyzing the first and third seconds of the responses, respectively (Nikonov, A. A. and Caprio, J., 2004). A reanalysis (Caprio, J., unpublished data) was performed of zebrafish OB unit responses over the initial 400 ms of the response as reported in Figure 3 of Friedrich R. W. and Laurent

(a) Antennular olfactory pathway



(b) Antennular nonolfactory chemosensory pathway



G. (2004) using the catfish scheme of identifying different groups of units by their selected excitatory responses to a specific type of amino acid. The results of this analysis suggested that 49 of the 58 units (84%) could be arranged into the same groups as had been reported for catfish – that is, those units that were preferentially excited by acidic, basic, short-chain neutral, and long-chain neutral amino acids. In addition, a fifth group emerged that was not detected in the channel catfish, those responsive to aromatic amino acids. Of these 49 zebrafish OB units, 63% were similarly classified when analyzing their responses over the first 1 s of the response, and 45% were similarly classified when the analysis time included 1.4 s of the response. These results suggest that there were not profound changes in responses of mitral cells in the zebrafish to the type of amino acid over response time and that the gradual dissolution of responses to types of amino acids over response time is possibly due to adaptational processes. For the zebrafish studies, however, the interpretation of the data is that responses of the population of mitral cells change over time in a stimulus-specific manner, thus providing a mechanism for the behavioral discrimination of individual amino acids (Friedrich, R. W. and Laurent, G., 2001). At present, the two different interpretations of the experimental results remain disputable.

Recently, the specificity of single OB units in the channel catfish to bile salts was reported (Rolen, S. H.

and Caprio, J., 2007). OB neurons were identified that were excited selectively by taurine-conjugated bile salts and nonconjugated bile salts, respectively. A third type of OB neuron was rather nonselective and was excited by at least one member of each of three types of bile salts: taurine-conjugated, glycine-conjugated, and nonconjugated.

4.04.4.2.5 Olfactory forebrain

The OB odotopic maps of the aforementioned species of teleosts provide a blueprint for investigation of the organization of odor processing at the next ascending synaptic level in the olfactory system. The axons of the output neurons of the OB (i.e., mitral and ruffed cells) constitute the medial and lateral OTs, which terminate in the cerebral lobes of the olfactory FB. A critical question is whether an odor map exists in the FB, and if so, whether its organization is based on odorant structure as it is in the OB and the antennal lobe of insects. An alternate possibility is that an FB map is not based on odorant quality but on odorant function (e.g., feeding or social odorant cues), or even, that due to the apparent extensive overlap of medial and lateral OT projections, a chemotopic map may not exist in the FB.

Recent experiments performed to visualize FB neurons in mice (Zou, Z. *et al.*, 2001; 2005) and mushroom body and lateral horn neurons of the protocerebrum in *Drosophila* (Wong, A. M. *et al.*, 2002; Marin, E. C. *et al.*,

Figure 3 Antennular chemosensory pathways in the brain of spiny lobsters. (a) Summary diagram of the olfactory (aesthetasc) pathway, which is a ventral view of the brain. The olfactory lobe (OL) receives input from aesthetasc olfactory receptor neurons (ORNs) via the main antennular nerve (A_1Nv). ORNs typically terminate in a single glomerulus (blue), though there may be multiglomerular ORNs (black). The OL has two general types of interneurons: projection neurons (PNs), with their somata in the lateral somata cluster (LC); and local neurons (LNs), with their somata in the medial somata cluster (MC). Three types of OL interneurons are shown, distinguished according to color (red, black, and gray). In red is a PN with multiglomerular innervation in the OL and an axon that bifurcates and extends via the olfactory globular tracts (OGTs) to higher-order neuropils. Two types of OL LNs are shown (purple and green). The accessory lobe (AL) is organized into three regions: medial lobe (ML), central lobe (CL), and lateral lobe (LL). The AL receives no direct sensory input from receptor neurons, but it does receive input from the OL via LNs. AL LNs (purple), similar to those of the OL, have somata in the MC. The AL has PNs (black and gray), which are similar to PNs of the OL in having somata in the LC and bifurcated processes that exit the brain via the OGTs. The deutocerebral commissure neuropil (DCN) and olfactory globular tract neuropil (OGTN) are also innervated by processes from OL interneurons. (b) Summary diagram of the nonolfactory (nonaesthetasc) pathway, which is a dorsal view of the brain. Nonaesthetasc antennular chemoreceptor neurons (CRNs) and antennular mechanoreceptor neurons (yellow) project via the antennular nerve (A_1Nv) into the ipsilateral LAN. The LAN has two regions: a lateral lobe and a medial lobe. There is a general topotopy, in that receptor neurons from the antennular lateral flagellum innervate the lateral lobe of the LAN, and receptor neurons from the antennular medial flagellum innervate the medial lobe of the LAN. These receptor neuron terminals overlap with arborization of the antennular motor neurons (red). The MAN receives mechanoreceptor input via the A_1Nv from the statocyst (blue), an organ for maintaining equilibrium. PNs of the LAN leave the brain via the protocerebral tract (PT). The tegumentary neuropil (TN) and antennal neuropil (AnN) receive sensory input (dashed black) from the second antenna (A_2Nv). (a) is adapted from Schmidt, M. and Ache, B. W. 1996b. Processing of antennular input in the brain of the spiny lobster, *Panulirus argus*. II. The olfactory pathway. *J. Comp. Physiol. A* 178, 605–628. (b) is adapted from Schmidt, M. and Ache, B. W. 1996a. Processing of antennular input in the brain of the spiny lobster, *Panulirus argus*. I. Non-olfactory chemosensory and mechanosensory pathway of the lateral and median antennular neuropils. *J. Comp. Physiol. A* 178, 579–604. Courtesy: Amy Horner.

2002) that receive input from specific glomeruli of the OB (mice) or antennal lobe (*Drosophila*) provided a consistent picture of the general organization of OT projection patterns and suggested the possible logic for olfactory information processing in these brain structures. Common to both mice and *Drosophila* was a stereotypic projection of secondary olfactory projection neurons from specific glomerular modules of the OB or antennal lobe that terminate with considerable overlap in their multiple brain target areas. The recent findings that projection classes with similar axon terminal fields in the lateral horn in *Drosophila* tended to receive input from neighboring glomeruli (Marin, E. C. *et al.*, 2002) and that structurally similar odorants in the honeybee (Sachse, S. *et al.*, 1999), zebrafish (Friedrich, R. W. and Korsching, S. I., 1997), and rodents (Tsuboi, A. *et al.*, 1999; Malnic, B. *et al.*, 1999; Strotmann, J. *et al.*, 2000; Uchida, N. *et al.*, 2000) likely activate adjacent and overlapping glomeruli of the OB or antennal lobe suggest an odotopic organization in these central olfactory processing centers. However, recent studies indicated that this map in higher-order olfactory centers is different from that within the OB or antennal lobe (Zou, Z. *et al.*, 2001; 2005; Marin, E. C. *et al.*, 2002; Wong, A. M. *et al.*, 2002). Thus, in contrast to the OB, axonal arborizations in FB are diffuse and extensive, and projections from different OB glomeruli often overlap to varying degrees. This anatomical organizational pattern suggests that third-order neurons in the olfactory pathway integrate odor information arriving from multiple OB glomeruli, which possibly codes for odorant quality and which may also relate to the odor's behavioral significance (e.g., food or pheromones) (Johnson, D. M. G. *et al.*, 2000; Haberly, L. B., 2001; Wang, Y. *et al.*, 2001; Zou, Z. *et al.*, 2001; Marin, E. C. *et al.*, 2002; Wong, A. M. *et al.*, 2002).

For channel catfish, an FB odotopic map resembling somewhat that present in the OB was recently determined (Nikonov, A. A. *et al.*, 2005; Nikonov, A. A. and Caprio, J., 2007). Both the medial–lateral distinction between excitatory responses to bile salts and amino acids and the rostrocaudal distinction between excitatory responses to amino acids and nucleotides reflect a similar topographical organization within the OB of the same species. The amino acid-responsive terminal field appears homologous to the olfactory cortex and perhaps the olfactory tubercle, whereas the bile salt-responsive region, the medial terminal field, is possibly homologous to portions of the amygdala in amniote vertebrates (Wullimann, M. F. and Rink, E., 2002; Wullimann, M. F. and Mueller, T., 2004; Nikonov, A. A. *et al.*, submitted). Furthermore,

as suggested by the previous anatomical investigations of possible convergence within the FB of OT fibers emanating from different OB glomeruli, cell types not previously observed within the OB were evident. These included units that were excited by both amino acids and nucleotides and others excited by both neutral and basic amino acids; however, there was no evidence of the convergence of odor information arriving via the separate medial and lateral OTs. Thus, convergence occurred between OT fibers providing input of food-related odorants, but not between food-relevant and socially relevant chemical signals.

4.04.4.3 Crustaceans

4.04.4.3.1 Organization of the olfactory lobes

CRNs of the antennules are packaged into a diversity of types of sensilla (see Section 4.04.3). One of these types is specialized as the olfactory sensilla – the aesthetascs. The ORNs of the aesthetascs project ipsilaterally to the paired OL (Schmidt, M. and Ache, B. W., 1996b) (Figure 3). The OLs are thought to receive input almost exclusively from aesthetasc ORNs (Schmidt, M. and Ache, B. W., 1992; 1996b; Sandeman, D. C. *et al.*, 1992).

The OLs have a glomerular organization (Figure 3), generally similar to first-order olfactory neuropils of other animals – the antennal lobes of insects and the OBs of vertebrates (Hildebrand, J. G. and Shepherd, G. M., 1997; Strausfeld, N. J. and Hildebrand, J. G., 1999; Eisthen, H. L., 2002; Schachtner, J. *et al.*, 2005). The neuronal components of the OL glomeruli are also generally similar to that of the olfactory neuropils of vertebrates and insects. Besides the primary afferent input from the aesthetasc ORNs, there are projection interneurons (PNs), several classes of local interneurons (LNs), and centrifugal neurons. The location of the somata associated with these neurons is precise: the LNs have their somata in the medial cluster (cluster 9), and the PNs have their somata in the lateral cluster (cluster 10). The OL glomeruli have clear subdivisions: cap, subcap, and basal regions. These regions contain different neuronal types and synaptic interactions (Schmidt, M. and Ache, B. W., 1996b; Wachowiak, M. *et al.*, 1997). Most ORNs branch in only one glomerulus, primarily in the cap and subcap regions (Schmidt, M. and Ache, B. W., 1992). PNs have multiglomerular projections, with dense innervation in a few glomeruli and sparse

innervation in many (Schmidt, M. and Ache, B. W., 1996b). The centrifugal fibers synapse on the somata of OL interneurons and are probably modulatory in function (Schmidt, M., 1997). The glomerular cap region is innervated by only two types of neurons: ORNs and multiglomerular GABAergic LNs. This implies that there are synapses between these neuronal types. This is probably the basis for the presynaptic inhibition from LNs onto ORNs (Wachowiak, M. *et al.*, 2002). Presynaptic inhibition in the OB also occurs in the vertebrates (Wachowiak, M. *et al.*, 2002).

Inhibition within the OL is mediated through at least two overlapping but functionally distinct inhibitory pathways (Wachowiak, M. and Ache, B. W., 1997). These are based on GABA and histamine, which shape the odor responses of OL units (Wachowiak, M. and Ache, B. W., 1998). These inhibitory circuits may have functional correlates with lateral inhibitory pathways in antennal lobe of insects and OB of vertebrates (Ache, B. W., 2002).

The number of OL glomeruli drastically varies across crustaceans, ranging from about 150 to 1300. The basis for this range is not obviously related to the number of aesthetascs, the animal's habitat, or phylogeny (Beltz, B. S. *et al.*, 2003).

Additionally, no clear sexually dimorphism in OLs is known, unlike has been described in some insects (e.g., the macroglomerular complex). This is in spite of the fact that sex pheromones are known in many crustaceans and their reception is mediated by aesthetascs (Gleeson, R. A., 1991; Kamio, M. *et al.*, 2002). It is possible that the OL possesses pheromone-specific glomeruli, but they are not morphologically distinct at a gross level.

Given findings that the glomerular organization in insects and vertebrates reflects odotopic mapping, it might be expected that the crustacean OL also has odotopic maps. There is indirect evidence for this, based on the fact that each aesthetasc expresses a diversity of ORNs (Steullet, P. *et al.*, 2000b) and the axons of ORNs from a single aesthetasc projects to many if not most glomeruli (Mellon, D. Jr. and Munger, S. D., 1990; Mellon, D. Jr. and Alones, V., 1993). The relationship between the number of ORNs and number of OL interneurons is instructive. For example, in spiny lobsters, each aesthetasc has ~300 ORNs (Derby, C. D. *et al.*, 2003). Aesthetascs appear to be functional units, with similar sets of ORNs in each aesthetasc (Steullet, P. *et al.*, 2000b); however, the number of different types of OR genes or ORNs is not known. Even assuming that there are

300 different types of ORs or ORNs, more than one type of ORN must innervate each glomerulus. However, direct tests of this hypothesis are lacking. Additionally, there is extensive convergence, given that there young adult spiny lobsters have about 300 000 ORNs per antennule projecting into 1200 glomeruli containing about 200 000 PNs and 100 000 LNs (Schmidt, M. and Ache, B. W., 1996b; Schachtner, J. *et al.*, 2005).

Closely associated with the OL, but lacking any primary sensory input, are the paired accessory lobes (ALs), olfactory globular tract neuropils, and deutocerebral commissure neuropils (Wachowiak, M. *et al.*, 1996; 1997; Sandeman, D. and Mellon, D., 2001; Ache, B. W., 2002; Schachtner, J. *et al.*, 2005) (Figure 3). The ALs are notable because of their large size in lobsters and crayfish and their glomerular organization. However, the AL glomeruli are smaller, more numerous, and of different synaptic organization than the OL glomeruli (Wachowiak, M. *et al.*, 1996; Schmidt, M. and Ache, B. W., 1996b). ALs have three regions – medial, central, and lateral – and each with specific synaptic connections (Wachowiak, M. *et al.*, 1996; Ache, B. W., 2002). For example, the medial and lateral regions have PNs, and the central region has interneurons connecting the AL to the OL as well as connection to the medial and lateral regions of the AL through LNs. The function of the AL is unknown, though its variance in size in different groups of crustaceans provides some grounds for speculation (Sandeman, D. C. *et al.*, 1993; Ache, B. W., 2002).

4.04.4.3.2 Organization of other chemosensory neuropils

All other antennular CRNs besides those in the aesthetascs, which include those on both the lateral and medial flagella of the antennules, project ipsilaterally to the paired LANs and to the unpaired MAN (Schmidt, M. and Ache, B. W., 1996a) (Figure 3). The LANs and MAN have a laminated organization, not glomerular. Unlike the OLs, the LANs and MAN also receive projections of mechanosensory neurons and antennular motor neurons. This organization is suggestive of topotopic maps, where space along the antennule is mapped topographically onto these neuropils (Schmidt, M. and Ache, B. W., 1993; 1996a; Schachtner, J. *et al.*, 2005); however, critical data are lacking.

4.04.4.3.3 Higher-order processing centers

The output interneurons from the OLs, ALs, and LANs project via the olfactory–globular tract (Figure 3) to a region of the protocerebrum called the terminal medulla and hemiellipsoid bodies, which are probably functionally equivalent to the mushroom bodies and lateral horn of insects (Schachtner, J. *et al.*, 2005). But the interneurons from the OLs, LANs, and ALs terminate in different regions of these neuropils, with phylogenetically diverse patterns of connection (Sullivan, J. M. and Beltz, B. S., 2004; 2005). The functional significance of these different projections and the activity of neurons in these higher-order neuropils are being explored (Sandeman, D. and Mellon, D., Jr. 2001; McKinzie, M. E. *et al.*, 2003).

4.04.4.3.4 Other chemoreceptor neuron processing centers

Virtually nothing is known about central processing of CRNs from the legs or mouthparts, except the identity of the regions into which their receptors project (e.g., Weissburg, M. J. *et al.*, 2001). While it is appreciated that the leg and mouthpart pathways control different behaviors than do the antennules (Derby, C. D., 2000; Horner, A. J. *et al.*, 2004; Garm, A. *et al.*, 2005), the mechanisms responsible for these differences remain largely unexplored.

4.04.4.4 Overview

The first-order olfactory processing center in both fishes and crustaceans, as with other vertebrates and arthropods, is glomerular in organization. These are the OB and AOB of fish, and the OL of crustaceans. The glomeruli are clusters of neuropil that contain the synapses between inputs (ORNs), outputs (mitral cells in fishes, projection neurons in crustaceans), and local interneurons. ORNs have ipsilateral projections, most being uniglomerular. The glomerular organization reflects an odotopy, with the odorants, at least food-related odorants, being represented across the glomeruli in a combinatorial fashion. Synaptic interactions within the OB and OL, which include inhibitory lateral connections, shape neuronal responses such that the output neurons of the OB and OL have different quality and temporal and response characteristics.

4.04.5 Neurogenesis and Turnover of Olfactory Neurons in Adult Crustaceans

Many crustaceans, fortunately including the model organisms for chemosensory research such as spiny lobsters, clawed lobsters, and crayfish, have indeterminate growth and add new ORNs to their olfactory organ and local and output interneurons to the OLs in the brain, throughout life including as adults (Steullet, P. *et al.*, 2000a; Harzsch, S., 2001; Derby, C. D. *et al.*, 2001a; Harrison, P. J. H. *et al.*, 2001a; Schmidt, M. 2001; Beltz, B. S. and Sandeman, D. C., 2003; Sandeman, R. and Sandeman, D., 2003).

Olfactory neurogenesis is highly flexible. Its rate can be modulated in adaptive ways by many variables. For example, following damage to the antennule, ORN neurogenesis increases dramatically so as to quantitatively compensate for the damage (Harrison, P. J. H. *et al.*, 2001a; 2003; 2004). As well, damage to the antennule modulates the rate of neurogenesis of olfactory interneurons (Sandeman, R. *et al.*, 1998; Hansen, A. and Schmidt, M., 2001; Sandeman, R. and Sandeman, D., 2003). Neurogenesis can also vary with internal factors, such as molt stage that is controlled by steroid hormones (Harrison, P. J. H. *et al.*, 2001a; 2001b), and environmental factors, such as circadian cycle (Goergen, E. M. *et al.*, 2002), season (Hansen, A. and Schmidt, M., 2004), environmental richness (Sandeman, R. and Sandeman, D., 2000), and social experience (Beltz, B. S. and Sandeman, D. C., 2003; Sandeman, R. and Sandeman, D., 2003; Song, C.-K. *et al.*, 2004).

In addition to continuous neurogenesis, olfactory neurons in the antennule, and to some extent in the brain, continuously turn over (Sandeman, R. and Sandeman, D. C., 1996; Steullet, P. *et al.*, 2000a; Schmidt, M., 2001; Harrison, P. J. H. *et al.*, 2001a; 2001b; 2003; 2004). The functional impact of this is that the olfactory organ completely turns over its ORNs after approximately four to five molts; the time for this depends on factors such as size of animal and environmental conditions, but for a young adult spiny lobster, this is approximately 1 year (Steullet, P. *et al.*, 2000a; Harrison, P. J. H. *et al.*, 2001a). The antennule is fully functional at any time, although the most proximal (newest) and distal (oldest) regions of aesthetascs have fewer aesthetascs and, at least in the case of the proximal (immature) aesthetascs, are not or less responsive to odors (Steullet, P. *et al.*, 2000a; 2000b). ORNs may be immature for several weeks or

more after their birth (Steullet, P. *et al.*, 2000a; Harrison, P. J. H. *et al.*, 2001a), and olfactory interneurons may be immature (defined by typical expression of neurochemicals) for several months (Schmidt, M., 2001).

The fact that the site of proliferation of new ORNs is in a very small area of the olfactory organ gives experimental advantages of using crustacean models over others in exploring aspects of olfactory neurogenesis. For example, candidate molecules involved in ORN proliferation can be identified by using techniques such as representational difference analysis to identify transcripts that are enriched in the region of ORN proliferation compared to regions of mature ORNs. Initial analyses of this kind have identified interesting candidates (Stoss, T. D. *et al.*, 2004). These include (1) one whose expression levels also increase following damage, which also upregulates neurogenesis; (2) a member of the same protein family as follistatin, which is an antagonist of GDF 11, and GDF 11 is expressed by progenitors of mammalian ORNs and whose absence prevents proliferation of ORNs (Wu, H.-H. *et al.*, 2003); and (3) a growth factor of the PDGF/VEGF family. RT-PCR approaches (Chien, H. *et al.*, 2005) have also identified *splash*, a spiny lobster homologue of achaete-scute genes, and which is expressed in regions of ORN neurogenesis in the olfactory organ. Since the mammalian achaete-scute homologue, MASH, is involved in differentiation of ORNs in rodents, *splash* is an interesting initial candidate in the exploration of molecular mechanisms of control and modulation of olfactory neurogenesis in crustaceans.

4.04.6 Taste in Fish

4.04.6.1 Peripheral Taste Anatomy

Taste buds in teleosts are located generally on the lips and within the oral cavity, including high densities on the gill rakers (Jakubowski, M. and Whitear, M., 1990; Sorensen, P. W. and Caprio, J., 1998; Finger, T. E., and Simon, S. A., 2000; Hansen, A. and Reutter, K., 2004) (Figure 4). The buds can be elevated on epidermal hillocks, be flush with the surrounding epidermis, or sunken. Taste buds are also found on the external body surface, primarily on the face and in some species, such as catfishes, along the entire external body surface (Reutter, K., 1978). This increase in extraoral taste buds is thought not to result directly in an increase in sensitivity but in a greater ability to localize a taste source (Bardach, J. E. *et al.*, 1967). Taste bud

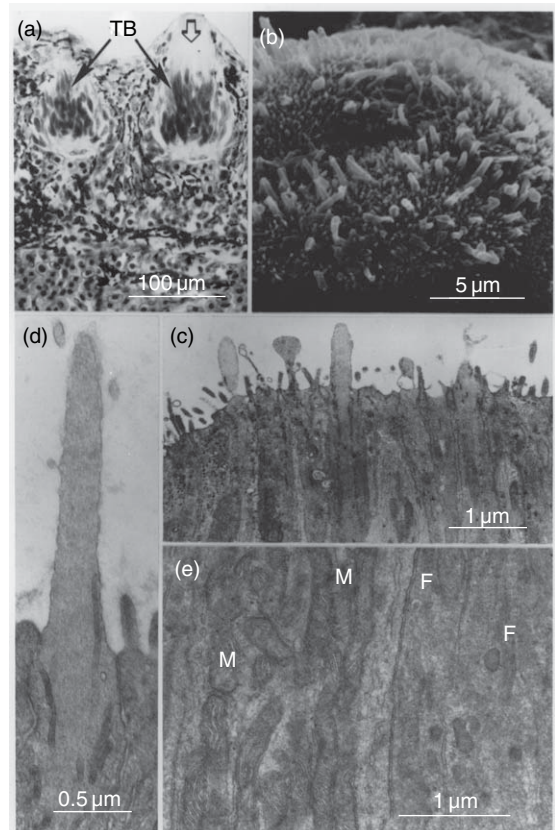


Figure 4 Teleost taste buds. (a) Light micrograph of a longitudinal section through two maxillary barbelle taste buds of the bullhead catfish, *Ameiurus nebulosus*. TB indicates two taste buds and cells within; these taste buds are of the elevated type (unfilled arrow). At the lower portion of each taste bud, basal cells are observed. (b) Scanning electron micrograph (SEM) of a portion of the surface of a catfish (*Ameiurus*) taste bud showing numerous small and fewer large receptor microvilli. (c) Transmission electron micrograph (TEM) of the apical portion of an *Ameiurus* taste bud in longitudinal section. The apical portion of the light cells forms a single large receptor villus of a light cell and a small villus of an adjacent dark cell in longitudinal section from a *Silurus glanis* (European Wels catfish) taste bud. (e) Detail of the supranuclear region of a longitudinally sectioned *Silurus* taste bud that shows numerous mitochondria (M) within a light cell (left) and bundles of intermediate filaments (F) in a dark cell (right). Reprinted from Sorensen, P. W. and Caprio, J. 1998. Chemoreception. In: The Physiology of Fishes (ed. D.H. Evans), pp. 375–405. CRC Press LLC, by courtesy of Taylor and Francis Group, LLC. Courtesy: Klaus Reutter.

densities can vary tremendously depending on the species, the location of the receptive field, and the size of the specimen. In some fishes, such as minnows and certain cyprinids, up to ~ 300 taste buds mm^{-2} were estimated (Gomahr, H. *et al.*, 1992). Teleost taste

buds contain up to ~ 100 cells that are generally divided into three types: light, dark, and basal cells (Reutter, K., 1978; Jakubowski, M. and Whitear, M., 1990). Light cells, considered the actual taste cell, possess a single large ($0.5\ \mu\text{m}$ thick, $1.5\text{--}3\ \mu\text{m}$ long) club-shaped microvillus at its apical surface, whereas dark cells, which partially wrap light cells, have at their apical surface numerous small ($0.1\text{--}0.2\ \mu\text{m}$ thick, $0.5\text{--}1.0\ \mu\text{m}$ long) microvilli and are considered supporting cells. However, dark cells in some species (e.g., ictalurid catfishes) form synapses with basal cells and may function also as taste cells (Reutter, K., 1978). Serotonin-rich basal cells, which resemble Merkel cells, number up to ~ 5 per taste bud, are oriented transversely to the longitudinal axis of the taste bud at the basal pole and form synaptic contacts with presumed taste cells. As in land vertebrates, taste cells undergo turnover. However, since teleosts are poikilothermic, the rate of turnover is dependent on the ambient water temperature. At temperatures of 14, 18, 22, and 30°C , taste cells in channel catfish have an average life span of 40, 30, 15, and 12 days, respectively (Raderman-Little, R., 1979). Also, as in other vertebrates, taste buds, depending on their location, are innervated by branches of either the VII, IX, or X CN. In addition to taste cells located within taste buds, teleosts can possess solitary chemosensory cells (SCCs), which resemble taste receptor cells that reach densities in excess of $100\,000\ \text{mm}^{-2}$ and, depending on the location on the fish, form synaptic contact with different afferent nerve fibers (V, VII, and spinal) (Kotrschal, K., 1991; 1993; Finger, T. E., 1997; Hansen, A. and Reutter, K., 2004). Furthermore, there can be considerable overlap between input to the CNS of both SCC and gustatory neurons as in rocklings *Ciliata* and *Gaidropsarus* (Teleostei: Gadidae), or the SCC input via spinal nerves can be quite distinct from the taste system as in searobins (*Prionotus carolinus*) (Finger, T. E., 1982; Kotrschal, K. and Finger, T. E., 1996). These anatomical differences in central connections suggest that the SCCs of rocklings and searobins are not homologous. Physiological studies on SCCs are scant, but they suggest that SCCs in rocklings respond primarily to body mucus of heterospecific fish (Peters, R. C. *et al.*, 1991).

4.04.6.2 Taste Cell Physiology and Transduction

The major class of taste stimuli studied in a wide variety of fish species is amino acids, although additional gustatory stimuli investigated in particular

species include small peptides, nucleotides, bile salts and acids, aliphatic acids, quaternary ammonium compounds, and steroids (Marui, T. and Caprio, J., 1992; Hara, T. J., 1993). High-affinity taste receptor sites for L-alanine (dissociation constant (K_D) of $1.5\ \mu\text{M}$) and L-arginine (two affinity states: K_D values of $18\ \text{nM}$ and $1.3\ \mu\text{M}$), respectively (Caprio, J., *et al.*, 1993), are located primarily on different taste cells within the same taste bud (Finger, T. E. *et al.*, 1996). The existence of independent gustatory receptor sites for L-alanine and L-arginine in channel catfish was determined previously from both electrophysiological and biochemical studies; a low-affinity receptor site for L-proline was also indicated (Caprio, J. *et al.*, 1993). Current evidence suggests that these three major classes of amino acid taste receptors in the channel catfish are coupled to activation of the taste receptor cells by two different mechanisms. At micromolar concentrations, L-alanine activates a G-protein-dependent increase in IP_3 or cAMP, but with a more rapid IP_3 production (Kalinowski, D. L. *et al.*, 1989). In contrast, both L-arginine and L-proline appear to be directly coupled to the activation of nonselective, but independent, cation channels (Kumazawa, T. *et al.*, 1998; Grosvenor, W. *et al.*, 2004). Further evidence of non-random expression of these amino acid taste receptors on taste cells is from electrophysiological studies of single taste fibers in the channel catfish, which has identified fiber types in the facial taste system that are highly responsive to L-alanine and L-arginine, respectively (Kohbara, J. *et al.*, 1992), and fiber types highly responsive to L-alanine, L-arginine, and L-proline, respectively, in the glossopharyngeal taste system (Ogawa, K. and Caprio, J., unpublished data).

Specific information concerning the molecular nature of taste receptors in vertebrates and the identification of their ligands has recently become available. As in mammals (Zhang, Y. *et al.*, 2003), fishes express two families of G-protein-coupled receptors, T1Rs and T2Rs in separate taste cells (Ishimaru, Y. *et al.*, 2005). T1Rs, members of class C GPCRs that possess an extensive N-terminus, exist as dimers. T1R dimers in fish detect amino acids (either T1R1/3 or multiple T1R2/3s), whereas T2Rs, which possess a short extracellular N-terminus and have not been shown to exist as dimers, detect bitter tastants (Oike, H. *et al.*, 2007).

4.04.6.3 Peripheral Nerve Taste Responses

Among the fishes tested electrophysiologically with amino acids, two groups were identified (Hara, T. J.

and Zielinski, B., 1989; Hara, T. J., 1993): (1) those whose facial taste system (those taste buds innervated by facial (CN VII) nerve fibers) responded to a number of different amino acids (i.e., wide response range) as demonstrated by channel catfish (Caprio, J., 1975; 1978) and (2) those whose facial taste systems were more selective (i.e., limited response range) as seen in salmonids (Hara, T. J. and Zielinski, B., 1989; Hara, T. J. *et al.*, 1999). Furthermore, multiunit recordings demonstrate that different branches of the facial nerve innervating different populations of taste buds located in different anatomical locations on the body of the fish have similar response properties and selectivities (Caprio, J., 1978; Davenport, C. J. and Caprio, J., 1982; Kanwal, J. S. *et al.*, 1987). The data are usually analyzed by comparing the magnitude of the integrated neural taste responses to the different chemicals at the same concentration. The relative magnitude of the integrated taste responses obtained even from different CNs can be similar as observed in rainbow trout (Kohbara, J. and Caprio, J., 2001). However, in other species such as channel catfish, taste responses between facial and glossopharyngeal/vagal nerves can be quite distinct (Kanwal, J. S. and Caprio, J., 1983; Kohbara, J. *et al.*, 1992). Facial taste thresholds for specific amino acids ranged generally between nano- and micromolar. Furthermore, depending on the species studied, taste thresholds of IX/X nerve fibers to specific amino acids can be either similar or higher than that determined for facial taste responses (Kanwal, J. S. and Caprio, J., 1983; Kohbara, J. and Caprio, J., 2001). Taste thresholds estimated from recordings from IX and/or X nerves in channel catfish were higher than those for VII, which is logical considering that high taste sensitivity of taste buds within the oropharyngeal cavity is not essential because of the higher stimulus concentrations present in the mouth once food intake occurs (Kanwal, J. S. and Caprio, J., 1987). The L-isomer of an amino acid was significantly more stimulatory than its D-isomer. However, a population of facial taste fibers in the sea catfish, *Arius felis*, was described that was more responsive to D- than to L-alanine (Michel, W. and Caprio, J., 1991) (see next section). In some fishes, sensitivity to a particular non-amino acid stimulus may even be greater than it is to amino acids. For example, the facial taste system of the rainbow trout to a bile salt, tauroolithocholate, approaches picomolar concentrations, which is 4 log units lower than for L-proline, the most stimulatory amino acid (Hara, T. J. *et al.*, 1984; Yamashita, S. *et al.*, 2006).

4.04.6.4 Processing Taste Information by Single Peripheral Taste Fibers

Since taste receptor cells are secondary receptors, they must synapse with afferent nerve fibers (VII, IX, or X) to transmit gustatory information to the primary gustatory nucleus of the medulla. A single taste fiber may synapse with multiple taste cells, not all of which are necessarily located in the same taste bud. For example, in channel catfish, the number of taste buds innervated by a single taste fiber of the recurrent facial nerve that innervates taste buds on the flank increases with the size of the fish, from two taste buds per axon in small fish (~5 cm in length) to nearly 14 taste buds per axon profile in larger fish (37–40 cm) (Finger, T. E. *et al.*, 1991). Information on the processing of taste information by peripheral nerve fibers in teleosts is extremely limited and is based primarily on quantitative analyses of responses of facial taste fibers in only a few species. The data obtained with amino acid stimuli from these species, however, indicate that there is sufficient similarity in the tuning characteristics of gustatory neurons to justify the existence of different fiber types.

Although single-unit studies of the taste specificity of teleosts were accomplished in only a few species of the more than 25 000 that exist, some tentative generalizations can be drawn:

1. Taste information concerning different classes of taste stimuli can be transmitted to the medulla by different groups (i.e., types of facial taste fibers); for example, the facial taste system of Japanese puffer fish (*Fugu pardalis*) consists of different populations of taste fibers with specificities to amino acids, nucleotides, and an inorganic acid, respectively (Kiyohara, S. *et al.*, 1985).
2. The specificity of a single taste fiber is not limited to a particular class of chemical stimuli; for example, one taste fiber type in the yellowtail flounder is highly responsive to both a nucleotide (uridine-5'-monophosphate) and an amino acid (L-tryptophan) (Zeng, C. and Hidaka, I., 1990).
3. Different taste fiber types having widely different tuning to members of the same class of chemicals can exist within the same CN of a particular species; for example, populations of facial taste fibers with different amino acid specificities occur in channel catfish (Kohbara, J. *et al.*, 1992; Caprio, J., *et al.*, 1993), sea catfish (Michel, W. and Caprio, J., 1991), Japanese puffer (Kiyohara, S. *et al.*, 1985), and yellowtail (Zeng, C. and Hidaka, I., 1990).
4. Taste fibers of different CNs (VII versus IX and X) within the same species can have different chemical

specificities; for example, a population of taste fibers highly responsive to L-proline occurs for the IX but not VII taste system in channel catfish (Ogawa, K. and Caprio, J., unpublished data).

5. The proportion and types of taste fibers comprising different CNs can vary within the same species; for example, the population of L-alanine taste fibers within the IX taste nerve in channel catfish was reduced by 55% compared with VII.
6. In addition to the existence of populations of taste fibers that are highly responsive to particular L-amino acids, taste fibers for D-amino acids may also exist; for example, 38% of the single facial taste fibers analyzed from the sea catfish, *Arius felis*, were most responsive to D-alanine (Michel, W. and Caprio, J., 1991). However, this fiber type also responded to L-alanine, and the L-alanine fiber type responded to D-alanine. High concentrations of D-amino acids occur in the tissues of soft-bodied invertebrates (Preston, R. L. 1987), potential prey items of the sea catfish. In contrast, only 3% of the single facial taste fibers in the freshwater channel catfish were most responsive to D-alanine (Kohbara, J. *et al.*, 1992).
7. Irrespective of the of the chemical specificity of the particular taste fiber types occurring in a species, stimulus quantity appears to be coded by the frequency of action potentials generated. For individual taste fibers, some have a dynamic response range of 5–6 log units of stimulus concentration, whereas other fibers, even in the same fish, have more truncated dose–response relations (Michel, W. and Caprio, J., 1991; Kohbara, J. *et al.*, 1992).
8. Some peripheral taste fibers in a particular species may be bimodal, that is, they respond to both taste and mechanical stimuli as in catfish (Davenport, C. J. and Caprio, J., 1982; Ogawa, K. *et al.*, 1997), whereas in other species, such as the Japanese puffer, this does not appear to be the case (Kiyohara, S. *et al.*, 1985).

4.04.6.5 Taste and Tactile Input to the Central Nervous System (Medulla)

Chapter 10 in this volume and a previous report (Kanwal, J. S. and Finger, T. E., 1992) provide excellent reviews of the gustatory pathways within the CNS of teleosts. This section is thus limited to a brief summary of the topographical manner in which taste and tactile information is represented in the primary gustatory nucleus of the medulla of catfishes since much of the limited information on the

processing of taste information was derived from these teleosts known for their elaborate gustatory system. Limited electrophysiological information also exists concerning taste and tactile activity in higher-order, that is, pontine (Lamb, C. F. IV and Caprio, J., 1992) and diencephalic (Lamb, C. F. IV and Caprio, J., 1993), gustatory nuclei in catfish but is beyond the scope of this review.

Taste and tactile information (see point 8 above) arriving via VII and IX/X terminates in register and in a somatotopic and viscerotopic manner, respectively, within the special visceral sensory column of the rostral medulla that is equivalent to the gustatory portion of the nucleus of the solitary tract in mammals. Both taste and tactile information from the external body surface and rostral oral cavity in catfishes is processed within the facial lobe (FL) of the medulla and that within the more posterior oral cavity and gill rakers by the glossopharyngeal nucleus (possibly a small lobe in some species) and vagal lobe (VL) (Finger, T. E., 1976; Kanwal, J. S. and Caprio, J., 1987). There is, however, a disproportionate representation of structures (e.g., an enlarged volume of medulla for the representation of barbels and gill rakers), which is indicative of their relative functional importance for taste and tactile input. The FL, through its input from external body taste buds and its interconnections to the spinal cord and medullary reticular formation, is involved in localization of a stimulus and controlling swimming behavior (Kanwal, J. S. and Finger, T. E., 1997). As such, the FL map is well defined and precise. The glossopharyngeal and vagal systems, which receive input from taste buds within the oral cavity, in contrast connects with brainstem nuclei in the control of swallowing and oropharyngeal movements (Atema, J., 1971; Finger, T. E. and Morita, Y., 1985). The VL map is not so important for the localization of food and is thus more diffuse than that of the FL (Kanwal, J. S. and Caprio, J., 1988).

For teleosts that possess specialized taste and tactile structures, such as barbels, the special visceral column can be subdivided into two to three lobes (Kanwal, J. S. and Finger, T. E., 1992). Within each lobe a finer representation of body parts (e.g., barbels and gill arches) can be represented in lobules (Kanwal, J. S. and Caprio, J., 1987; Hayama, T. and Caprio, J., 1989; Kiyohara, S. and Caprio, J., 1996). For catfishes, taste and tactile inputs from each of the different barbels project to a different lobule within the FL (Kiyohara, S. *et al.*, 1986; Kanwal, J. S. and Caprio, J., 1988; Marui, T. *et al.*, 1988). In addition to

the existence of peripheral bimodal (taste and tactile) fibers, mechanosensory input into the primary gustatory nucleus also occurs via mechanosensory-only facial (Davenport, C. J. and Caprio, J., 1982; Kiyohara, S. *et al.*, 1985) and trigeminal (Kiyohara, S. *et al.*, 1986) fibers. Medullary neurons responsive to taste stimuli (i.e., amino acids) were limited to smaller areas of the electrode tracks than neurons sensitive to touch alone (Marui, T., 1977; Marui, T. and Funakoshi, M., 1979; Marui, T. and Caprio, J., 1982; Kanwal, J. S. and Caprio, J., 1988). The overlap of taste and tactile maps is interesting from a neuroethological aspect as both types of input are involved in the detection and selective food ingestion in catfish and are likely processed simultaneously prior to activation of the respective motor neurons.

4.04.7 Processing of Mixtures

4.04.7.1 Introduction

In all natural environments, including aquatic, olfactory and gustatory receptor cells rarely encounter single pure chemicals as are usually presented in experimental studies. The composition of many biologically relevant mixtures in aquatic environments, particularly of food, is well characterized (Carr, W. E. S., 1988). Behavioral studies demonstrate the high potency of animal extracts and mixtures of amino acids to fishes and crustaceans. Additionally, however, the magnitude of the response to a chemical mixture is poorly predicted from knowledge of the responses to the individual components – revealing mixture interactions. These are either mixture suppression or mixture enhancement, in which the response to the mixture is less or greater, respectively, than expected. Obviously then, defining the expected response to a mixture becomes the critical issue. To do so for a CRN, it is critical to know several of its features: Does a CRN have both excitatory and inhibitory transduction pathways? Does a CRN have more than one type of receptor? If so, how are these coupled to the excitatory and/or inhibitory transduction pathways? Which mixture components interact with each receptor and transduction pathway? How do those components interact with the receptors – that is, are they agonists or antagonists, and is their action competitive or noncompetitive?

The more completely characterized is the cell, the more accurate is the predicted response to a mixture. In other words, mixture interactions are a consequence of an incomplete understanding of the above processes.

Fish and crustaceans, especially catfish and spiny lobsters, serve as model organisms to understand how mixtures are processed. These studies are built largely on electrophysiological and biochemical studies that have defined the diversity of receptor types and transduction pathways at the cellular level. Arguably, our understanding of how the sensory systems of animals detect, process, and ultimately lead to the discrimination and perception of mixtures is as complete for catfish and lobsters as it is for any animal. (And sadly, we still have a long way to go!) This section summarizes our understanding of mixture processing in these systems.

4.04.7.2 Fish

4.04.7.2.1 Introduction

Electrophysiological studies in teleosts attempted to predict the global response, that is, olfactory EOG (electro-oculography) and olfactory and gustatory integrated neural activity (Hidaka, I. *et al.*, 1976; Caprio, J., *et al.* 1989; Kohbara, J. and Caprio, J., 1996; Ogawa, K. and Caprio, J., 2000) to simple (binary and ternary) mixtures of amino acids. Additionally, a single olfactory electrophysiological investigation studied responses to complex mixtures (up to 10 components) of amino acids (Kang, J. and Caprio, J., 1991). The overall results of these investigations indicated that knowledge of the relative independence of the receptors for the component stimuli in a mixture, obtained from receptor binding and/or electrophysiological cross-adaptation experiments, was essential to successfully predict the magnitude of the electrophysiological response. Mixtures whose components showed little competition or cross-adaptation generated responses greater than those whose components were indicated to compete with a common receptor or with receptors having highly overlapping specificities. There was little evidence for mixture suppression. That mixture interactions are weak in the peripheral olfactory system in teleosts was also indicated in the zebrafish (Tabor, R. *et al.*, 2004). For olfaction, where single ORNs express one or at most a few ORs (Ngai, J. *et al.*, 1993; Mombaerts, P. 2004), the greater response to a mixture of amino acids was likely due to the activation of multiple populations of ORNs, each ORN within a specific population expressing one of the receptors for a particular component in the mixture. The greater response to mixtures observed in the peripheral taste system was also likely due to the activation of different receptors wherever expressed; for this effect observed in single taste fibers in the channel catfish (Ogawa, K. and Caprio, J., 1999) and Japanese puffer (Hidaka, I. *et al.*,

1976), the different receptors could theoretically be located on individual cells within a single taste bud or on different taste cells in different taste buds innervated by the single taste fiber. These studies also indicated for both olfactory and gustatory systems that the greater responses to mixtures are more likely due to the simultaneous activation of different receptors by the individual components of the mixture than by stimulus binding at the same total concentration to a single receptor. The greater response to a mixture whose components bind to relatively independent receptors than to a single or highly cross-reactive receptors may be the electrophysiological correlate of the behavioral observations that mixtures are often more stimulatory than individual components, even in cases when the concentration of the individual component is higher than the total concentration of the multiple-component stimuli within the mixture.

4.04.7.2.2 Responses of single units

4.04.7.2.2.(i) Olfaction There are few quantitative physiological studies of how single olfactory neurons in fishes respond to stimulus mixtures – primarily amino acids. For teleosts, only in the channel catfish was a quantitative study of single ORNs performed to stimulus mixtures (Kang, J. and Caprio, J., 1997), whereas responses of single OB neurons to stimulus mixtures were investigated in both the channel catfish (Kang, J. and Caprio, J., 1995b; 1997; Tabor, R. *et al.*, 2004) and zebrafish (Tabor, R. *et al.*, 2004). For channel catfish, the types of responses of single ORNs and OB neurons to binary mixtures comprising components that were either both excitatory, both suppressive, or both non-responsive were similar (based on response type) to those of the components in 80% (for ORNs) and 82% (for OB neurons) of the trials. These results are comparable to a similar study in rats where 75% of the responses of single OB neurons to binary mixtures evoked similar response patterns as the components of the binary mixtures (Giraudet, P. *et al.*, 2002). Overall, these results indicate that profound mixture interactions are rare in simple mixtures where the components evoke similar responses.

For simple binary mixtures where the responses to the components were different (i.e., excitatory (E) and nonresponsive (N); suppressive (S) and N; E + S, respectively), responses of catfish OB neurons to the mixture were often similar in 94% of the trials to that for one of the components (Kang, J. and Caprio, J., 1995b). For example, in a binary mixture of an excitatory and a suppressive component, the response of an OB neuron to the mixture was either excitatory or

suppressive in 94% of the trials – in only 6% of the trials was the mixture nonresponsive. For catfish ORNs, responses to the mixtures were similar to that for one of the components in 82% of the trials for E + S mixtures and 100% for both E + N and S + N mixtures (Kang, J. and Caprio, J., 1997). In a comparable study in the OB of the rat, units evoked a similar pattern of response, as did one of the components in the mixture in 83% of the trials (Giraudet, P. *et al.*, 2002). For zebrafish OB units, where binary mixtures of amino acids comprised an excitatory and a suppressive component ($n = 18$), the response to the binary mixture was dominated (in 85% of the cases) by the excitatory response, whereas the suppressive component occurred in the remaining 17% of the trials. In additional experiments, the component responses and those to the binary mixtures were complex, differing primarily in their temporal patterns (Tabor, R. *et al.*, 2004). In contrast, for OB units in channel catfish where excitatory and suppressive responses to the mixture components tested were equivalent (29%; 42% null), the response to the binary mixture was excitatory in 34%, suppressive in 41%, and null (no response significantly different from control) in 25% of the trials ($n = 32$) (Kang, J. and Caprio, J., 1995b). Mixture suppression or masking, where a component reduced or concealed the neuron's excitatory or inhibitory response to the other component, however, was observed with single ORN and OB neurons in catfish and single OB neurons in rat. This effect was observed when there was no significant response to a binary mixture composed of an effective stimulus (evoking either an E or S response) and a nonstimulatory component. Such mixture suppression occurred in 62% (for E + N components) and 68% (for S + N components) of these trials in catfish ORNs, 43% in catfish OB neurons, and 46% in rat OB neurons (when analyzing firing rates).

4.04.7.2.2.(ii) Taste Electrophysiological responses of integrated and single-unit facial taste responses to binary mixtures of amino acids in the channel catfish (Ogawa, K. and Caprio, J., 1999) were consistent with previous olfactory results obtained in the same species (see above). These reports collectively indicate that the magnitude of multiunit responses to binary and more complex olfactory and gustatory stimulus mixtures is greater if the component stimuli bind to relatively independent receptor sites than to the same or highly cross-reactive sites. The study also indicated that the greater taste activity observed to the mixture was not significantly different, whether

recording the taste activity in a multifiber or a single-unit preparation. This finding is noteworthy in indicating that greater taste activity is not exclusively the result of the components in a binary mixture simultaneously activating different fiber types (i.e., having different chemical specificities); that is, the greater response also occurred by the activation of different taste receptors on taste cells innervated by the same single taste fiber. Also indicated was that the magnitude in enhanced taste activity could be significantly different across the different responding gustatory fiber types. In the Japanese puffer, *F. pardalis*, the amino acid derivative betaine (*N*-trimethyl glycine) enhanced taste activity of facial taste fibers to particular amino acids (Hidaka, I. *et al.*, 1976).

4.04.7.2.3 Behavioral discrimination

Fish are similar to humans in that components of binary mixtures are discriminable. Humans can identify up to approximately three components in either taste (Laing, D. G. *et al.*, 2002) or olfactory mixtures (Laing, D. G. and Francis, G. W., 1989). Whether a binary mixture of amino acids is detected by fishes as a unique odor or whether the qualities of the individual components are retained within the mixture was investigated in catfish (Valentinčič, T. *et al.*, 2000a). It was previously determined for catfish that the discrimination of chemicals is based on olfaction and not on taste (Valentinčič, T. *et al.*, 1994). Catfish conditioned to a binary mixture initially treat the mixture as the component eliciting the greater physiological response (based on the magnitude of the EOG in response to each component). This result is similar to that for humans, where binary odor mixtures are perceived as the more stimulatory component (Laing, D. G. and Willcox, M. E., 1983). Additional discrimination training in catfish, however, facilitates the discrimination of the less potent component in a binary mixture – that is, the binary mixture is no longer detected as its more stimulatory component. Thus, with enough training, catfish are able to elementally process mixtures (i.e., identify the separate components of the mixture).

4.04.7.3 Crustaceans

4.04.7.3.1 Electrophysiology

Electrophysiological studies of processing of mixtures by crustaceans have been performed exclusively using single cells rather than global responses such as EOGs. Thus, explanations are possible at the single-cell level

as well as at the system level through analysis of populations of these single cells.

Single CRNs of crustaceans have multiple transduction pathways, including excitatory and inhibitory pathways and including more than one receptor type in the excitatory pathway (see Section 4.04.2). Thus, when trying to understand the response to a mixture, even a simple one such as a binary mixture, the magnitude and direction of the response (i.e., increase or decrease from spontaneous spiking rate) will depend on whether the components of a mixture activate the excitatory or the inhibitory pathway and on whether they activate the same or different receptor sites in a given pathway. As a consequence, responses to mixtures can be either more or less than the more excitatory component. The following are some examples.

If a CRN has both excitatory and inhibitory transduction pathways, and one chemical activates the excitatory pathway and another chemical activates the inhibitory pathway, then we would expect that a mixture of these two chemicals would generate a response less than excitatory chemical and greater than the inhibitory chemical. This has been observed from intracellular whole-cell patch clamp recording from lobster CRNs (Michel, W. C. and Ache, B. W., 1992). This type of effect can explain some cases of mixture suppression identified from extracellular measurement of spiking activity of single-unit recordings from lobster CRNs. These CRNs often have very low levels of spontaneously spiking activity – often much less than 1 spike per second – so that if a compound activates an inhibitory transduction pathway, this inhibition is not expressed as a reduction in spiking activity. If only spiking activity were being recorded, this would lead to an identification of mixture suppression. Yet knowing the transduction cascades activated by these components of a mixture leads to a predicted response that matches that observed.

If a CRN has two receptors types, each coupled to an excitatory transduction pathway as seems to be the case for many CRNs of crustaceans (see Section 4.04.2), then it is important to know how the components of a mixture interact with these receptor types. The predicted responses are different if the mixture components bind primarily to the same receptor or bind to different receptors. If the components compete for the same receptor, then whether they are competitive agonists or antagonists will influence the response generated by the mixture. The clearest demonstration of this is in a study by Cromarty S. and Derby C. (1997). This study examined responses

of taurine-sensitive CRNs to binary mixtures of excitatory compounds, either competitive agonists (taurine and structural analogues of taurine) or non-competitive agonists (taurine and structurally dissimilar molecules). Responses to binary mixtures of competitive agonists were significantly lower than responses to mixtures of noncompetitive agonists, but they were exactly that expected from a competitive model. Knowing how these compounds bind to receptors and how these receptors are coupled to transduction cascades allowed accurate predictions of responses to mixtures; without this, mixture interactions would be suggested to have occurred.

As described in the next section, behavioral studies suggest that spiny lobsters can use elemental processing to analyze mixtures, such that the mixture is perceived as a set of identifiable components (Livermore, A. *et al.*, 1997). This behavioral finding has implications on how mixtures are processed by the peripheral olfactory system. It suggests that a mixture and its components should generate different neural response profiles and that the response profile of the mixture should have features in common with its components. In addition, if one component of a mixture dominates the perception of a mixture, then the neural profile generated by the mixture should be more similar to that of the dominant component. These ideas were tested using different blend ratios of binary mixtures (Steullet, P. and Derby, C. D., 1997). The results suggest that for the neural profiles generated in the peripheral olfactory system of spiny lobsters, the qualities of individual compounds are maintained when the compounds are mixed to form blends. This result suggests a neural explanation of the ability of spiny lobsters to elementally process an odor mixture.

4.04.7.3.2 Behavior

Mixture interactions have been examined at the behavioral level in some detail in several crustacean species, including the glass shrimp *Palaemonetes pugio* (Carr, W. E. S. and Derby, C. D., 1986a; 1986b), the Caribbean spiny lobster *P. argus* (Derby, C. D. *et al.*, 1989; Daniel, P. C. and Derby, C. D., 1991), the California spiny lobster *Panulirus interruptus* (Zimmer-Faust, R. K. *et al.*, 1984; Zimmer-Faust, R. K., 1987; 1993), and the American lobster *H. americanus* (Borroni, P. F. *et al.*, 1986; Atema, J. *et al.*, 1989). In these studies, typically the magnitude of the behavioral response (usually an appetitive response) to a mixture is compared to that of the responses to that mixture's components. The response magnitudes are then

compared to each other and to the predicted responses calculated on a variety of models that fall into two classes: (1) those that assume the components interact with the same transduction pathways and (2) those that assume the components activate independent transduction pathways. The outcomes of these studies differ. For the glass shrimp, mixture enhancement or synergism, in which the mixture response was significantly greater than expected, was common. Compounds that by themselves had no activity, when mixed with each other or with compounds that were moderately active, generated very strong responses. Using an antennular flicking assay on the Caribbean spiny lobster and binary mixtures of compounds, mixture suppression was much more common, in which the response to a binary mixture was less than expected. In the California spiny lobster, adding a repellent compound (ammonium) to an attractive compound (ATP) predictably produced a response to the mixture that was less than that to the attractant alone (Zimmer-Faust, R. K. *et al.*, 1984; Zimmer-Faust, R. K., 1987; 1993). From these results, it is clear that predicting responses to mixtures based only on responses to components is unreliable. We have argued earlier that with detailed knowledge of the transduction pathways within an ORN and the chemical stimuli that activate each of them, accurate predictions of mixture responses are possible. But of course for behavior, many neuronal levels exist between ORNs and motor output, including complex synaptic interactions. For example, some mixture interactions occur at the level of the CNS, independent of the ORNs (Derby, C. D. *et al.*, 1985). Thus, generating a better understanding of processing of chemical information in the CNS is essential for understanding the behavior of animals to mixtures.

The ability of lobsters to discriminate among chemical stimuli is known from studies using aversive paradigms similar to those used on other animals. Such associative conditioning generates an aversive response to one chemical stimulus, even one previously attractive, and then animals can be examined for response generalization to other stimuli (Fine-Levy, J. B. *et al.*, 1988). This procedure was used for several sets of stimuli that were also used in electrophysiological studies of mixture processing (see below). These include single compounds; binary mixtures that differ only in blend ratios, and their components; and complex mixtures of 29–35 components that mimic their natural food (crab, shrimp, oyster, and mullet). These results show that lobsters show the greatest generalization between stimuli that are closest in composition. For example,

lobsters show much more generalization between crab mixture and shrimp mixture than between other mixtures, and this is highly correlated with the degree of similarity in the blend ratios of these mixtures (Fine-Levy, J. B. *et al.*, 1988; 1989; Fine-Levy, J. B. and Derby, C. D., 1991).

Behavioral studies also showed that spiny lobsters perceive chemical mixtures in different ways, depending on the salience of the components. They can learn them as a set of elemental cues if the salience of the components during learning is sufficiently high. In addition, they can also learn them as a configural cue (i.e., as a mixture-unique entity) if the salience of the individual components is not emphasized (Livermore, A. *et al.*, 1997; Derby, C. D., 2000).

4.04.7.4 Overview

Catfish and spiny lobsters are two of the more intensely studied animals regarding the neural processing and behavioral responses to chemical mixtures, and as such, they provide excellent case studies. Behavioral studies show that both catfish and spiny lobsters perceive the components of binary and even larger mixtures as discriminable elements, if the salience or relevance of those components is emphasized. This implies elemental processing. If the salience of the components is not emphasized, then mixtures are perceived as a configural (i.e., mixture-unique) cue. This leads to the conclusion that each discriminable component of a mixture should generate a unique neural profile, at least at early processing levels. Electrophysiological studies on catfish and lobsters support this conclusion. Individual studies identified examples of mixture interactions, in which the response to the mixture is different from that expected from the response to the mixture's components. However, the body of work on catfish and spiny lobsters demonstrates that the more completely characterized is a cell or a system, the more accurate is the predicted response to a mixture. If one knows the receptor types, the transduction cascades, how receptor types are coupled to those cascades, and which chemical stimuli interact with each receptor type and transduction cascade, then by using rigorous experimental procedures, responses to mixtures are highly predictable. A few studies on catfish and lobsters demonstrate this (Caprio, J., *et al.*, 1989; Kang, J. and Caprio, J., 1991; Cromarty, S. and Derby, C., 1997). The challenge is of course to build a detailed

understanding of all of these molecular pathways. Since mixtures are the biologically relevant stimuli for animals, this is more than a worthy endeavor, as difficult as it is.

4.04.8 Behavioral Roles of Olfaction and Taste

4.04.8.1 Introduction

The chemical senses are involved to some degree in many behaviors of fishes and crustaceans. These include finding food, mates, habitat, and shelter, avoiding predators, social interactions, and homing. Although many studies investigated the chemosensory behavior of different species to chemical stimuli, the vast majority did not determine which chemosensory systems – olfaction, taste, or others – were primarily responsible for the behavioral results. Since both olfactory and taste systems for particular species of fishes and crustaceans can be highly sensitive to specific classes (e.g., amino acids) of chemical stimuli (Caprio, J., 1978; Goh, Y. and Tamura, T., 1980; Hara, T. J., 1994), without the appropriate controls, it can be confusing as to which system or systems are responsible for releasing a specific behavioral response.

4.04.8.2 Fish

Olfaction is involved in the detection and search for food and is uniquely implicated in the detection of pheromones and other chemicals that provide information concerning sex, social interactions, alarm, and homing. Taste, on the other hand, is also associated with food-related behaviors, but those that are more of a reflexive nature, such as ingestion, biting, and swallowing of food; for catfishes, taste also participates directly in the localization of the food source (Hara, T. J., 1993; Sorensen, P. W. and Caprio, J., 1998).

Typical feeding stimulants of fishes are amino acids, and both the olfactory and gustatory systems are generally highly stimulated by amino acids. However, the chemical specificity and the specific functions of the two chemosensory systems to these compounds can be quite distinct (Caprio, J., 1977; Goh, Y. and Tamura, T., 1980; Kohbara, J. *et al.*, 1992; Nikonov, A. A. and Caprio, J., 2004), and the feeding behaviors controlled by the two are not identical but are overlapping. For a generic fish (i.e., one without an elaborate extraoral taste system) such as rainbow trout, both olfactory (Hara, T. J., 1973) and gustatory (Marui, T. *et al.*, 1983) systems can be highly sensitive to amino acids. However, anosmic

rainbow trout are incapable of initiating feeding in response to these chemical cues, in spite of a fully functioning taste system (Valentinčič, T. and Caprio, J., 1997). In contrast, for catfishes, which possess extensive extraoral and oral taste systems and a concomitant increase in the neural circuitry of the medulla facial and VLs to process gustatory information, both food search and ingestion are released in anosmic specimens (Bardach, J. E. *et al.*, 1967; Valentinčič, T. and Caprio, J., 1994). Olfaction in catfishes, however, is essential for the discrimination of different amino acid stimuli in a learning paradigm (Valentinčič, T. *et al.*, 1994; 2000b; Valentinčič, T., 2004).

4.04.8.3 Crustaceans

There are differences, as well as some overlap, in those behaviors controlled by olfaction (aesthetascs) and taste. In spiny lobsters, the antennules drive detection of and orientation to distant, attractive chemicals, including food-related chemicals and intraspecific chemicals such as aggregation pheromones and alarm pheromones (Derby, C. D. *et al.*, 2001b; Horner, A. J. *et al.*, 2004; Shabani, S. and Derby, C. D., 2006) and pheromones (aggregation cue and alarm cues) (Horner, A. J. and Derby, C. D., 2005; Shabani, S. and Derby, C. D., 2006). Interestingly, either the aesthetasc (olfactory) pathway alone or the nonaesthetasc antennular pathway alone is sufficient to mediate the animal's response to food odors (Horner, A. J. and Derby, C. D., 2005; Shabani, S. and Derby, C. D., 2006). In addition, both of these pathways can mediate discrimination of and learning about food-related chemicals, be they single compounds, simple mixtures, or complex mixtures. Thus, there is considerable redundancy between the aesthetasc and the nonaesthetasc antennular pathways. However, differences in the functions of the aesthetasc and nonaesthetasc antennular pathways have been identified as well. For example, only the aesthetasc pathway, and not the nonaesthetasc antennular pathway or extra-antennular chemosensory pathway, mediates attraction to aggregation pheromones or alarm pheromones (Horner, A. J. *et al.*, 2004; Horner, A. J. and Derby, C. D., 2005; Shabani, S. and Derby, C. D., 2006). "Aesthetascs are also necessary for the response of clawed lobsters to social odors (Johnson, M. E. and Atema, J., 2005)". Thus, the aesthetascs uniquely possess detectors of aggregation and alarm cues.

It must be emphasized that although we have placed the nonaesthetasc antennular sensors in a single category, they differ not only structurally but also functionally. On the antennular flagella, there are nine

different types of nonaesthetasc sensilla (Cate, H. S. and Derby, C. D., 2001). Although they all are bimodally innervated by chemosensory neurons and mechanosensory neurons that project to the LANs, they differ in setal structure and organization and in location on the antennule (Cate, H. S. and Derby, C. D., 2001; 2002). They also differ in function. For example, the asymmetric sensilla are uniquely responsible for mediating chemically activated antennular grooming behavior (Schmidt, M. and Derby, C. D., 2005).

Chemosensors on the legs of lobsters differ structurally and functionally from antennular sensory. In lobsters, they direct local searches to food chemicals, and when the leg touches the source of the chemicals, that item is grabbed and transferred to the mouthparts (Derby, C. D. and Atema, J., 1982).

Mouthpart chemoreceptors, with their high threshold and broad tuning (Garm, A. *et al.*, 2005), control whether food should be eaten (Derby, C. D. and Atema, J., 1982). Presumably they are checking the quality of the prey, such as determining whether feeding stimulants are present and deterrents are absent.

The hierarchy of control of behaviors by the different chemosensors as described above for lobsters may not apply completely to all crustaceans. For example, in the blue crab *Callinectes sapidus*, leg chemoreceptors can also play a role in orientation to distant cues (Keller, T. A. *et al.*, 2003).

Crustaceans demonstrate several types of learning involving their chemical senses, including habituation and aversive associative learning (Derby, C. D., 2000), one-trial flavor avoidance learning (hermit crab), and other important behaviors though less well-defined mechanistically (Caldwell, R. and Dingle, J., 1985; Wight, K. *et al.*, 1990). Aversive associative learning has been used to study spiny lobster's ability to discriminate chemical stimuli, including single-odorant compounds and different blend ratios of the same binary or complex mixtures (Derby, C. D., 2000). The associative task is learning to avoid a previously attractive food stimulus. Since this task is controlled by the antennules, expression of this learned task depends on the presence of fully functional antennules. But it does not require aesthetascs: either the aesthetasc (olfactory) pathway or the nonaesthetasc antennular pathway can mediate discrimination and learning about chemical stimuli (Steullet, P. *et al.*, 2001; 2002).

4.04.8.4 Overview

The chemical senses of fish and crustaceans control or modulate many of their behaviors. There are overlaps

and differences in the behaviors that are mediated by the different chemosensory systems, including olfaction and taste. Both olfactory and nonolfactory systems can mediate orientation to distant food-related chemicals. Olfactory systems tend to control detection of pheromones and thus cues associated with intraspecific interactions. Some of the behaviors controlled by olfaction include learning about chemicals involved in discrimination. Taste and other nonolfactory systems are uniquely associated with chemically activated biting and swallowing of food and other more reflexive behaviors.

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