

Chapter 7

Neuronal Processing of Chemical Information in Crustaceans

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Abstract Most crustaceans live in aquatic environments and chemoreception is their dominant sensory modality. Crustacean chemoreception is mediated by small cuticular sense organs (sensilla) occurring on all body parts, with the antennules (first antennae), second antennae, legs, and mouthparts representing the major chemosensory organs. Chemoreceptive sensilla of crustaceans are divided into *bimodal sensilla* which comprise a few mechano- and some chemoreceptor neurons and occur on all appendages and *aesthetascs* which are innervated by 40–500 olfactory receptor neurons and exclusively occur on the antennular outer flagellum. Olfactory receptor neurons differ from chemoreceptor neurons of bimodal sensilla in having spontaneous activity, inhibitory responses, and autonomous bursting, but both types of receptor neurons mainly respond to small water-soluble molecules such as amino acids. The dichotomy in sensilla structure is reflected in the organization of the associated CNS pathways. Olfactory receptor neurons selectively innervate a synaptic region in the midbrain, the olfactory lobe, which is organized into dense substructures called glomeruli. As is typical of the first synaptic relay in the central olfactory pathway across metazoans, olfactory information processing in glomeruli is based on multiple types of inhibitory local interneurons and on projection neurons ascending to higher brain areas. Receptor neurons from bimodal sensilla target synaptic areas that are distributed throughout the brain and ventral nerve cord and contain arborizations of motoneurons innervating muscles of the segmental appendages that provide the chemo- and mechanosensory input. Based on the matching dichotomy of sensilla construction and of sensory pathway organization, we propose that crustacean chemoreception is differentiated into two fundamentally different modes: “olfaction” – chemoreception mediated by the aesthetasc–olfactory lobe pathway, and “distributed chemoreception” – chemoreception mediated by bimodal sensilla on all appendages and the associated synaptic areas serving as local motor centers. In decapod crustaceans, pheromone

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detection and processing of pheromone information are not mediated by dedicated sensilla and CNS pathways, respectively, but seem to be integral components of olfaction and distributed chemoreception. Aesthetascs mediate responses to distant pheromones, whereas bimodal sensilla located on the appendages touching the conspecific partner are likely responsible for the detection of contact pheromones.

List of Abbreviations

AL	Accessory lobe
CNS	Central nervous system
CRN	Chemoreceptor neuron
dCRN	“Distributed” chemoreceptor neuron of bimodal sensillum
DC	Deutocerebral commissure
DCN	Deutocerebral commissural neuropil
LAN	Lateral antennular neuropil
LN	Local interneuron
MAN	Median antennular neuropil
MRN	Mechanoreceptor neuron
OGT	Olfactory globular tract
OGTN	Olfactory globular tract neuropil
OL	Olfactory lobe
ORN	Olfactory receptor neuron (CRN of aesthetasc)
PN	Projection neuron

7.1 Introduction

For most crustaceans, chemoreception is the dominant sensory modality. My (MS) interest in crustacean chemoreception started when I realized, during my diploma thesis, that most sensilla of crustaceans contain chemoreceptor neurons and occur in many different types on all appendages. Since then, trying to understand how behavior is controlled by these sensilla has been a cornerstone of my research. I (DM) have been fascinated by crayfish and their behaviors since, as a young boy, I would capture them in a creek near my home and keep them in an aquarium in my bedroom. We have both since then gone long ways trying to unravel the neuronal basis of chemoreception.

Numerous behavioral studies have shown that chemoreceptors in crustaceans occur on all appendages and many parts of the body. Among these, the antennules (first antennae), second antennae, legs, and mouthparts are the major chemosensory organs (Brock 1926; Spiegel 1927; Hindley 1975) (Fig. 7.1). Based on the perceived duplicity of chemical senses in vertebrates, several attempts were made to

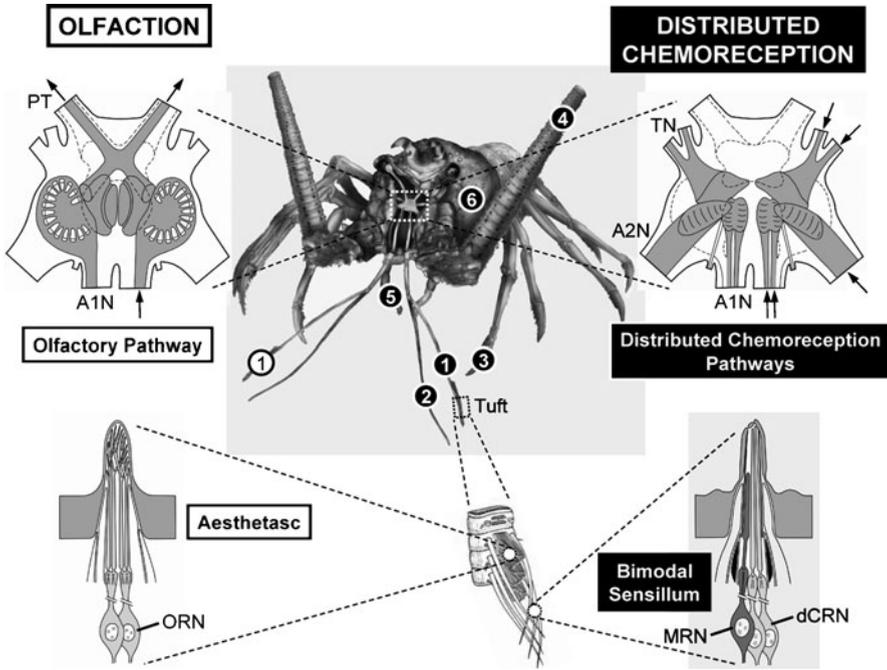


Fig. 7.1 Overview of chemoreception in decapod crustaceans. *Center*: Artists’ drawing of spiny lobster showing the location of chemoreceptive sensilla (*white dot*: aesthetascs; *black dots*: bimodal sensilla) on different body parts (*1*: lateral flagellum of antennule; *2*: medial flagellum of antennule; *3*: walking legs; *4*: second antenna; *5*: third maxillipeds; *6*: gill chamber). Tuft region of lateral flagellum shown in higher magnification (modified from Grünert and Ache, 1988). Lobster drawing by Jorge A. Varela Ramos. *Left*: Organization of “Olfaction.” *Bottom*: Ultrastructure of aesthetasc (ORN: olfactory receptor neuron) (modified from Schmidt and Gnatzy, 1984); *Top*: Olfactory pathway in the brain with arrows indicating direction of information flow (AIN antennular nerve; PT protocerebral tract to lateral protocerebrum in eyestalk ganglia). *Right*: Organization of “Distributed Chemoreception.” *Bottom*: Ultrastructure of bimodal sensillum (MRN mechanoreceptor neuron; dCRN “distributed” chemoreceptor neuron) (modified from Schmidt and Gnatzy, 1984); *Top*: Distributed chemoreception pathways in the brain with arrows indicating sensory inputs (A2N antennal nerve; TN tegumentary nerve)

categorize crustacean chemoreception into “olfaction” and “taste.” For crustacean chemoreception this distinction has been based on differences in sensitivity (Laverack 1968), on differences in the elicited behaviors and the appendages mediating them (Atema 1980), and finally on how chemoreception is interwoven with mechanoreception (Laverack 1988). However, none of these attempts fully matches the available data and none has found general acceptance. We propose that chemoreception in crustaceans in fact comprises multiple chemical senses and that the distinction of only two – “olfaction” and “taste” – failed because it is incomplete.

7.2 Olfaction and Distributed Chemoreception

Crustaceans detect chemicals with small cuticular sense organs called sensilla. Crustacean chemoreceptive sensilla – in spite of their diversity in outer structure – fall in two main classes: (a) *Bimodal sensilla* are innervated by 1–3 mechanoreceptor neurons (MRNs) and 1–22 chemoreceptor neurons (CRNs) whose unbranched dendrites run in a narrow canal to a terminal pore at the tip of a thick-walled hair shaft (seta); (b) *Aesthetascs* are innervated by 40–500 CRNs whose branched dendrites fill the wide lumen of a thin-walled tube-like seta (Schmidt and Gnatzy 1984; Hallberg and Skog, Chap. 6) (Fig. 7.1). Bimodal sensilla of numerous structural types occur on the body and all appendages and in their construction correspond to insect contact chemoreceptors. Aesthetascs are morphologically homogeneous, exclusively occur on the outer flagellum of the antennules, and represent olfactory sensilla corresponding to those of insects in ultrastructure. Hence, we will use the term olfactory receptor neurons – ORNs – for the CRNs they contain.

The dichotomy in the structure of crustacean sensilla is reflected in the organization of the associated sensory pathways within the CNS. The crustacean brain consists of three paired ganglia, the protocerebrum receiving input from the compound eyes, the deutocerebrum receiving input from the antennules, and the tritocerebrum receiving input from the second antennae and the head (Sandeman et al. 1992). The aesthetasc ORNs selectively innervate a neuropil (= region where neurons interact via synaptic contacts) of the deutocerebrum, the olfactory lobe (OL). The OL is organized into glomeruli (= small clumps of particularly dense neuropil) which are characteristic of the first synaptic relay of the central olfactory pathway across higher metazoans (Sandeman et al. 1992; Schachtner et al. 2005) (Figs. 7.1 and 7.2b). The glomerular organization of the OL contrasts with a striated (perpendicular to the long axis) organization of the neuropils receiving input from bimodal sensilla, the second antenna neuropils, the lateral antennular neuropils (LAN), and the leg neuromeres (Tautz and Müller-Tautz 1983; Schmidt and Ache 1996a) (Figs. 7.1 and 7.2a). The perpendicular striation appears to result from a topographical representation of sensory input and an interdigitating arborization pattern of motoneurons driving the muscles of the segmental appendage providing the sensory input. Thus, these neuropils act as local motor centers of the segmental appendages and mediate direct sensory-motor integration.

The matching dichotomy of sensilla construction and neuroanatomical organization of sensory neuropils suggests that in crustaceans chemical information is received and processed in two fundamentally different modes. One mode is “Olfaction” which we define as chemoreception mediated by the aesthetasc – OL pathway; the second mode is “Distributed Chemoreception,” which we define as chemoreception mediated by bimodal sensilla on all appendages and the associated striated neuropils that serve as local motor centers. Distributed chemoreception not only comprises “taste,” which we define as contact chemoreception in the context of

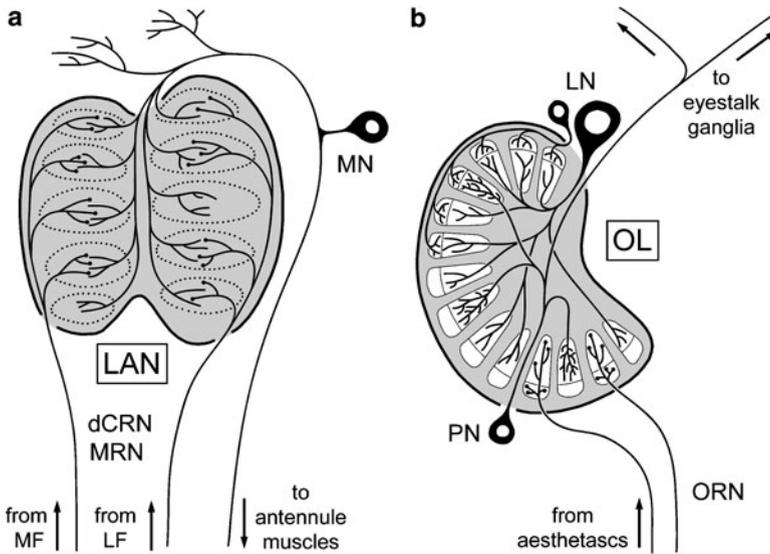


Fig. 7.2 Schematic representations of chemosensory pathways in the brain. (a) Lateral antennular neuropil (LAN) pathway. dCRNs and MRNs from sensilla on the lateral (LF) and medial (MF) antennular flagellum project somatotopically into both lobes of the LAN and couple directly to antennular motoneurons (MN). (b) Olfactory pathway. ORNs project nontopographically into the olfactory lobe (OL) which is organized into glomeruli. Information is processed by various types of multiglomerular local interneurons (LN) and output is provided by multiglomerular projection neurons (PN) ascending to the lateral protocerebrum in the eyestalk ganglia

food detection and control of ingestion, but also other “chemical senses” with specialized function, like the control of antennular grooming (Schmidt and Derby 2005) and the coordination of mating and copulation (Belanger and Moore 2006; Bauer, Chap. 14; Snell, Chap. 23).

Behavioral studies have shown that particular chemically-elicited behaviors are mediated exclusively by certain sensilla types. Aesthetascs mediate courtship behavior in response to distant sex pheromones, agonistic interactions, aggregation behavior, and avoidance behavior in response to conspecific alarm cues (Shabani et al. 2008, and references therein). Asymmetric setae – bimodal sensilla neighboring the aesthetascs (Fig. 7.3a–d) – mediate antennular grooming in response to chemical stimulation (Schmidt and Derby 2005). On the other hand, aesthetascs and bimodal sensilla on the antennules both contribute to complex chemically-elicited behaviors such as food search (Steullet et al. 2001; Horner et al. 2004) and associative odor learning (Steullet et al. 2002). This suggests that different sensilla types not only serve specific behavioral functions and thus likely differ in their physiological properties, but also that different sensilla types participate in the control of complex behaviors indicating that they have overlapping functional properties.

7.3 Physiological Methods Used for Analyzing Crustacean Chemoreception

The electrophysiological analysis of crustacean chemoreceptors is mainly based on extracellular recordings from bundles of receptor neuron axons which usually do not allow attributing axon activity to a specific sensillum type. This is particularly problematic for the analysis of ORNs. Their axons are extremely thin (diameter 0.1–0.3 μm ; Spencer and Linberg 1986; Mellon et al. 1989) and intermingle with thicker axons from neighboring bimodal sensilla (Fig. 7.3e–g) making it more likely that the latter are the source of discriminable action potentials in the antennular nerve. Only the ORNs of the aesthetascs of *P. argus* have been studied by patch clamp recordings from their somata (pioneered by Anderson and Ache 1985) leaving no ambiguity about the origin of the responses. The processing of

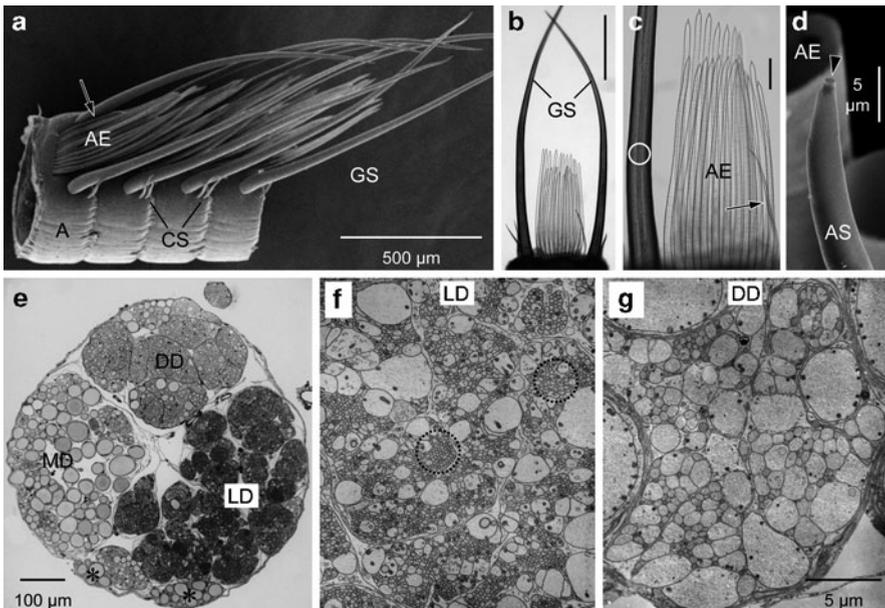


Fig. 7.3 Morphology of crustacean chemoreceptive sensilla and sensory nerves. (a – d) Tuft region of the lateral flagellum of *P. argus* (scale bars: B = 500 μm , C = 100 μm). Each annulus (A) bears two rows of aesthetascs (AE) which are associated with three types of bimodal sensilla: long guard setae (GS) with a thin central canal (circle in c), small companion setae (CS), and one asymmetric seta (AS) (arrow in a and c) with a terminal pore (arrowhead in d). (e–g) Organization of the antennular nerve of *P. argus* (f, g: TEM micrographs at the scale shown in g). (e) The antennular nerve is organized into a lateral division (LD) containing axons of sensilla on the lateral flagellum, a dorsal division (DD) containing axons of sensilla on the medial flagellum, a medial division (MD) containing axons of sensilla on the basal segments, and two bundles of motoneuron axons (asterisks). (f) Axon bundle in the lateral division contains numerous very thin ORN axons (dotted circles) mixed with axons of larger diameter. (g) Axon bundle in the dorsal division contains axons of various sizes. (a, d) from Schmidt and Derby 2005; (e) from Schmidt et al. 1992

chemosensory input in the CNS of crustaceans has been investigated using intracellular recordings and labeling of the recorded neurons by intracellular markers (Ache and Sandeman 1980; Arbas et al. 1988; Wachowiak and Ache 1994; Mellon and Alones 1995; Schmidt and Ache 1996a, b). Most of the available knowledge is from the antennular-deutocerebral pathway of three species of decapod crustaceans, the spiny lobster *P. argus*, and the crayfish *P. clarkii*, and *Cherax destructor*.

7.4 Physiology of Pheromone Reception in Crustaceans

Most of what is known about crustacean chemoreception is in the context of food detection and ingestion; only very little is known in other behavioral contexts, such as predator avoidance, orientation in the habitat, interactions with symbiotic partners, or intraspecific communication by pheromones. From recent behavioral and neuroanatomical studies, two important concepts about pheromone reception in crustaceans have emerged:

(A) In crustaceans, as insects, pheromones act on two distinct spatial scales, distance and contact. This dichotomy is well established for sex pheromones, some of which mediate distant attraction while others control copulatory behavior upon body contact (e.g., Kamiguchi 1972; Kamio et al. 2002; Bauer, Chap. 14). Responses to distant pheromones (mating, aggregation, agonistic interactions, individual recognition) or alarm cues released by conspecifics are mediated by the aesthetascs (Shabani et al. 2008, and references therein). Behavioral observations suggest, sensilla mediating responses to contact pheromones are located on the appendages touching the conspecific partner, usually the second antennae, and/or the legs (Bauer, Chap. 14) and hence must be bimodal sensilla.

(B) In decapod crustaceans, neither the sensilla mediating responses to pheromones nor the pathways in the CNS that are implicated in processing pheromone information show specializations indicating that pheromones are detected or processed differently than “ordinary” chemical stimuli. This is obvious in sex pheromone detection where such specializations would be apparent as sex-specific differences in sensilla endowment and/or the neuroanatomy of sensory pathways in the brain as found in many insects. In decapods, sex-specific differences in the number of aesthetascs and of bimodal sensilla on the second antennae only occur in few species of shrimp with males having more sensilla than females (Kamiguchi 1972; Bauer, Chap. 14). However, sex-specific sensilla types or sex-specific differences in the organization of neuropils receiving chemosensory input have not been reported for any decapod.

7.5 Physiology of Chemoreceptor Neurons

Chemosensitivity has been electrophysiologically confirmed for all behaviorally identified major chemosensory organs of crustaceans, namely, the antennules (both flagellae), second antennae, legs, and mouthparts (Caprio and Derby 2008) (Fig. 7.1).

7.5.1 *Location of Bimodal Sensilla and Aesthetascs*

Bimodal sensilla have been identified on both flagella of the antennules (Cate and Derby 2001, 2002), on the *second antennae* (Voigt and Atema 1992), on the third maxillipeds and other mouthparts (Derby 1982; Garm and Hoeg 2006), and on the distal leg segments in particular the dactyl including – in clawed crustaceans – the chelae (Derby 1982; Altner et al. 1983; Schmidt and Gnatzy 1984). In contrast, aesthetascs only occur on the lateral flagella of the antennules. In *P. argus*, *Panulirus interruptus*, and *H. americanus*, in which most electrophysiological studies were performed, 1,000–1,500 aesthetascs form a regular distal tuft on the lateral flagellum (Figs. 7.1 and 7.3a–c). Each aesthetasc is innervated by approximately 300 ORNs whose somata form a dense cluster below the aesthetasc seta (Spencer and Linberg 1986; Grünert and Ache 1988; Cate and Derby 2001). The aesthetascs are associated with several types of bimodal sensilla, among them are: guard setae, companion setae, and asymmetric setae (Fig. 7.3a–d) (Cate and Derby 2001, 2002).

7.5.2 *Response Properties of dCRNs and ORNs*

To determine how different CRNs respond to chemical stimuli, the following response properties are determined: (1) general firing properties including spontaneous activity, direction of response (excitation or inhibition), and rhythmic activity, (2) coding of stimulus quality measured as specificity and spectral tuning in response to different single chemical compounds, and (3) coding of stimulus intensity measured as sensitivity in response to different concentrations of one chemical compound.

(A) Bimodal sensilla: dCRNs of bimodal sensilla have very little or no spontaneous activity and respond with an increase in the frequency of action potentials, i.e., an excitation, to chemical stimulation (Figs. 7.4a and 7.6a, c). Typically, dCRNs have a phasic-tonic temporal response profile characterized by a strong and transient excitation at the onset of a stimulus (phasic component) and a lower excitation maintained throughout the stimulus duration (tonic component). Some dCRNs, however, respond purely tonic or purely phasic (e.g., Derby and Atema 1982a, b; Hatt and Bauer 1982; Schmidt and Gnatzy 1989; Garm et al. 2005). In addition to dCRNs, bimodal sensilla contain 1–3 MRNs which typically respond directionally to touch or water movements (Derby 1982; Altner et al. 1983; Cate and Derby 2002).

To analyze the spectral tuning of dCRNs, food extracts or mixtures of chemicals mimicking such extracts have been used as “search” stimuli and this limits our knowledge about stimulatory chemicals for crustacean dCRNs to mainly food-related substances. The chemicals most widely tested and found to be stimulatory are L-amino acids followed by ammonium chloride, betaine, nucleotides, amines, sugars, pyridines, organic acids, and small peptides. Only rarely, other chemicals, including the putative pheromone ecdysone (Spencer and Case 1984), were tested

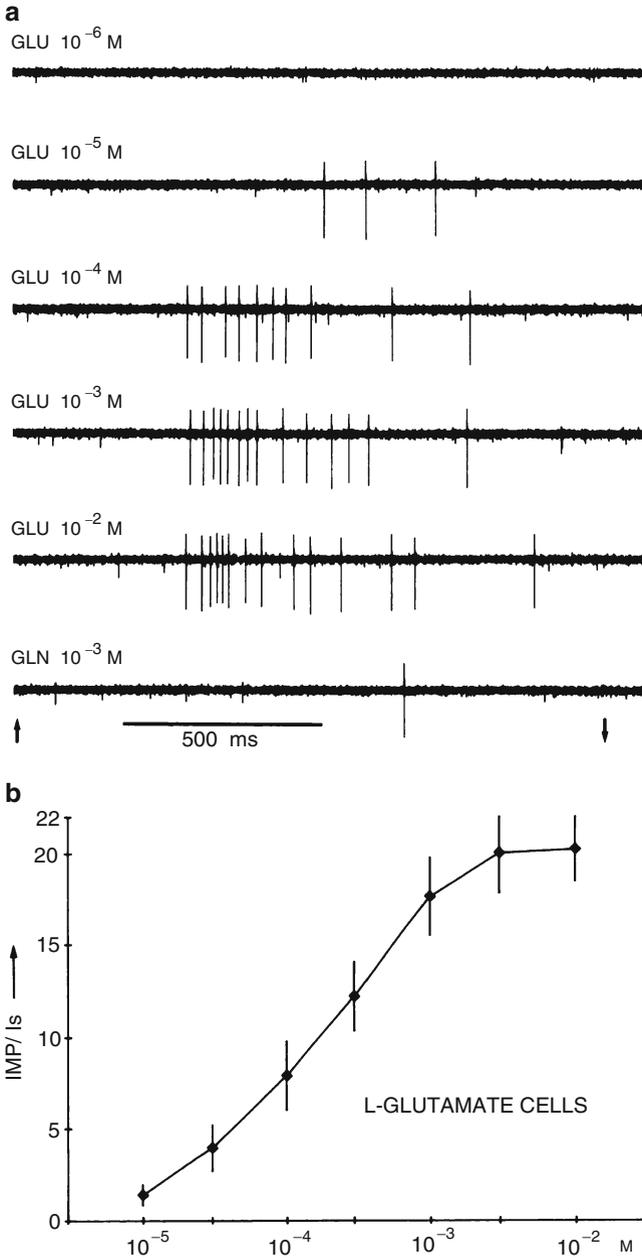


Fig. 7.4 Physiological properties of dCRNs on the legs (in funnel-canal organs of the shore crab, *C. maenas*). **(a)** Responses of an L-glutamate-best cell to stimulation with ascending concentrations of L-glutamate. **(b)** Mean dose-response curve of 13 L-glutamate-best cells. The working range (3×10^{-5} to 3×10^{-3} M) indicates that under natural conditions these cells only respond when contacting food. From Schmidt and Gnatzy 1989

and found to activate dCRNs. In breadth of tuning, dCRNs range from very narrowly tuned responding strongly to only one chemical to more broadly tuned responding strongly to several chemicals (e.g., Tierney et al. 1988; Schmidt and Gnatzy 1989; Corotto, Voigt and Atema 1992; Voigt and Atema 1992; Voigt et al. 1997; Garm et al. 2005) (Fig. 7.5). Between different chemosensory appendages, dCRNs do not differ systematically in breadth of tuning.

Determination of the sensitivity of dCRNs to single chemicals revealed that they mostly have threshold concentrations (minimal concentration eliciting a significant response above control) between 10^{-9} and 10^{-6} M for the most stimulatory chemicals with a working range (concentration range between threshold and saturation) of 2–3 orders of magnitude (Derby and Atema 1982a, b; Hatt and Bauer 1982; Tierney et al. 1988; Voigt and Atema 1992) (Figs. 7.4b and 7.6b, d). However, some dCRNs located on the legs as well as on both flagella of the antennules have either significantly higher (5×10^{-6} – 5×10^{-4} M) or lower ($<10^{-12}$ M) threshold concentrations (Thompson and Ache 1980; Derby and Atema 1982a; Carr et al. 1986; Schmidt and Gnatzy 1989). dCRNs located on mouthparts generally have a high threshold concentration of 10^{-6} – 10^{-5} M and a working range of about 3 orders of magnitude (Corotto et al. 1992; Garm et al. 2005).

(B) Aesthetascs: The general response properties of ORNs have three unique features: (1) most ORNs show spontaneous tonic activity ranging from 0.1 to ca. 8 Hz, (2) ORNs are excited by some and inhibited by other chemical stimuli indicating that two transduction pathways are present in each ORN, and (3) some ORNs (ca. 30%) show spontaneous, rhythmic bursts of action potentials (Fig. 7.7) (Anderson and Ache 1985; Michel et al. 1991; Michel et al. 1993; Bobkov and Ache 2007).

The specificity of single ORNs has not been analyzed systematically. In two studies, almost all tested amino acids caused excitatory responses in some ORNs but L-proline, L-arginine, and L-cysteine also elicited inhibitory responses in many ORNs (Michel et al. 1991, 1993) (Fig. 7.7b). Since the same amino acid excited some but inhibited other ORNs, the response type (excitation or inhibition) was not a property of the stimulant but depended on the ORN.

The sensitivity of ORNs has not been analyzed systematically. One study found that the threshold concentration of some amino acid-sensitive ORNs ranged from 10^{-6} to 10^{-4} M with a working range of 1–3 orders of magnitude (Michel et al. 1993) suggesting that ORNs may be less sensitive than CRNs of neighboring bimodal sensilla.

7.6 Projections of Antennular Chemoreceptors in the Brain

Axons from chemo- and mechanosensory sensilla on the antennular flagella course within branches of the antennular nerve to three major target areas in the deutocerebrum: The olfactory lobes (OL), the lateral antennular neuropil (LAN), and the median antennular neuropil (MAN). Backfilling techniques in spiny lobsters have established that ORNs connect with synaptic targets exclusively within the OL, while dCRN and MRN axons target the LAN and MAN (Schmidt et al. 1992;

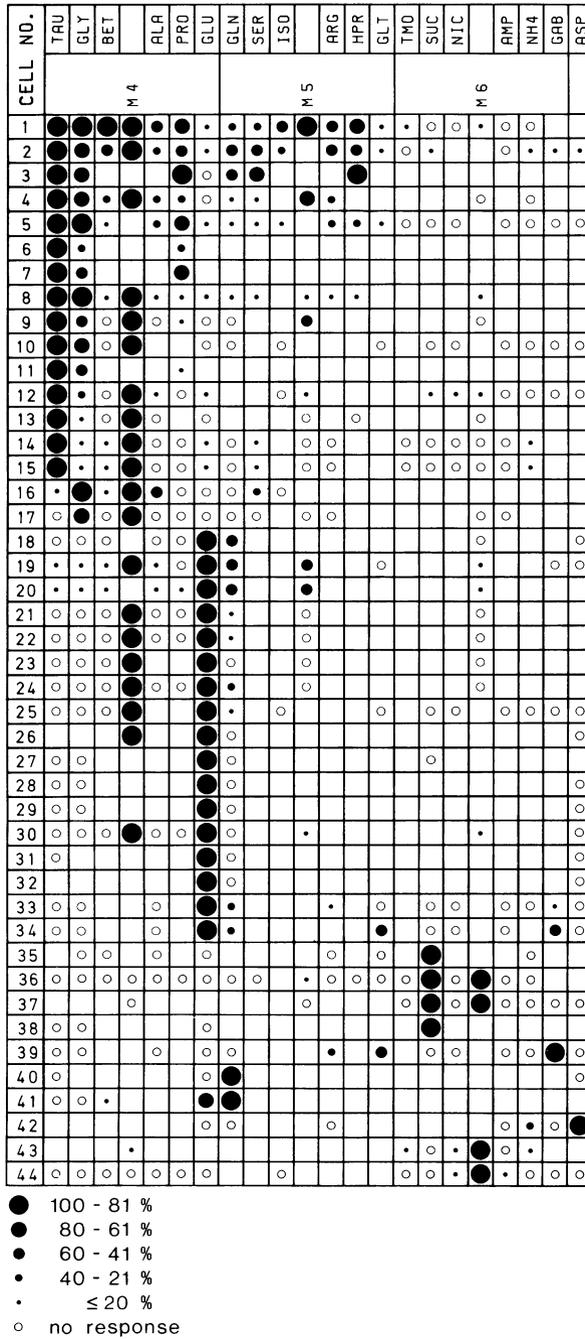


Fig. 7.5 Physiological properties of dCRNs on the legs (in funnel-canal organs of the shore crab, *C. maenas*). Response spectra of 44 cells to stimulation with 19 single chemicals. Some dCRNs are broadly tuned but most are narrowly tuned with L-glutamate-best and taurine-best cells being most frequent. From Schmidt and Gnatzy 1989

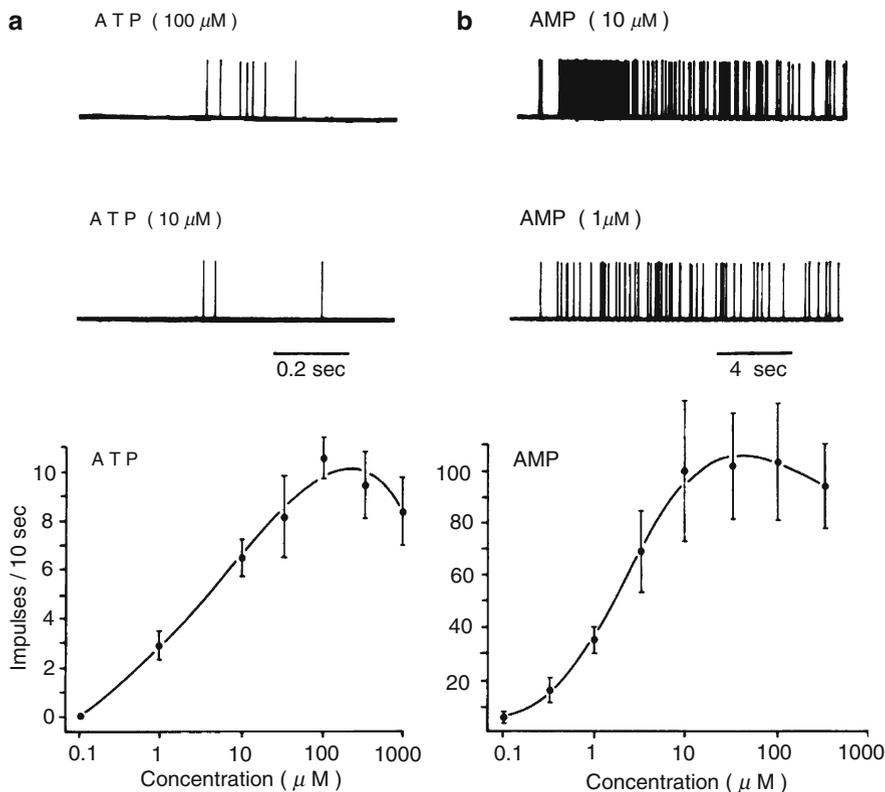


Fig. 7.6 Physiological properties of dCRNs on the antennules. Responses of an ATP-best (a) and an AMP-best cell (c) on the lateral flagellum of *P. argus* and mean dose–response curves of 11 ATP-best cells (b) and 7 AMP-best cells (d). The working range of these cells (10^{-7} – 10^{-4} M) indicates that under natural conditions they may not respond to distant stimuli. From Carr et al. 1986

Schmidt and Ache 1992) (Fig. 7.2a, b). In crayfish the innervation of neither the MAN nor LAN has been specifically examined, but ORN axons have been successfully traced to the OL (Sandeman and Denburg 1976; Mellon et al. 1989; Mellon and Munger 1990; Mellon and Alones 1993; Sandeman and Sandeman 1994). In spiny lobsters and crayfish, ORN axons penetrate individual OL columnar glomeruli from the periphery, arborize extensively in the peripheral cap region, and a few branches run axially to the base of the columns, making terminal and *en passant* synaptic connections along the way (Figs. 7.2b and 7.10). Electron microscopical studies in the crayfish OL suggest that ORN axons form synapses with higher-order neurons primarily in the cap region, whereas synaptic connections between higher-order neurons predominate in the subcap and base (Mellon and Alones unpublished observation).

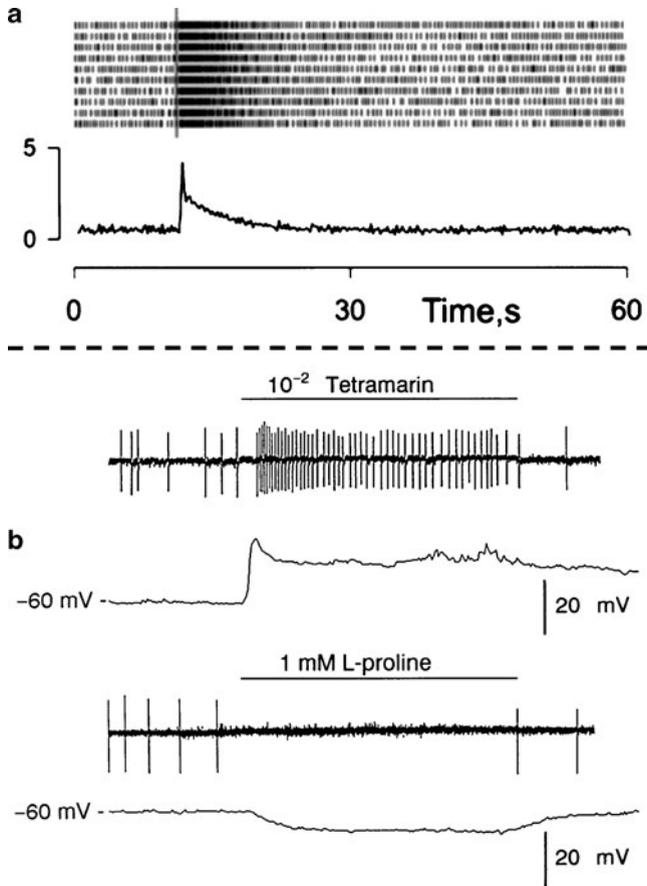


Fig. 7.7 Physiological properties of ORNs in aesthetascs of *P. argus*. (a) Responses of an ORN to repetitive stimulation with a food extract and average response (number of spikes/200 ms). Note the ORN-typical high spontaneous activity. (b) Coexistence of excitatory and inhibitory responses in a single ORN (top traces – action potentials, bottom traces – membrane potential). The spontaneously active ORN is excited by stimulation with a food extract (Tetramarin) but inhibited by stimulation with L-proline. (a) from Bobkov and Ache 2007; (b) from Michel et al. 1991

Current interpretations of ORN projections across taxa suggest that each ORN expresses just one type of receptor protein, and all ORNs expressing identical receptor proteins on their dendrites terminate within the same glomerulus. In mammals, each class of ORNs (about 1,000) which expresses the same receptor protein on its dendrites, terminates in a specific glomerulus, or pair of glomeruli, in the olfactory bulb (Munger et al. 2009); this principle, however, may not apply as strictly to insects or to other arthropods.

7.7 Central Olfactory Pathways in Crayfish and Spiny Lobster

7.7.1 Organization of Deutocerebral and Projection Pathways

In both crayfish and spiny lobsters, in addition to the paired OLs, the deutocerebrum comprises the paired LANs and the unpaired MAN as well as paired accessory lobes (ALs), olfactory globular tract neuropils (OGTNs), and deutocerebral commissural neuropils (DCNs). While paired ALs are present in crayfish, spiny lobsters, and clawed lobsters, they are highly reduced in size in brachyurans and anomurans, and they are totally absent in the Stomatopoda, the Dendrobranchiata, the Caridea, and the Stenopodidea (Sullivan and Beltz 2004). The AL is organized into small glomeruli and does not receive primary sensory inputs of any modality. It does, however, receive secondary or tertiary multimodal inputs from several sensory systems, including the olfactory system, the visual system, and somatosensory systems (Sandeman et al. 1995; Wachowiak et al. 1996; Mellon 2000). These findings are consistent with a role for higher order multimodal processing in the AL. A deutocerebral commissure (DC) connects the two ALs; its axons terminate bilaterally in AL glomeruli. In *P. argus* local interneurons interconnect the OL with the AL (Wachowiak and Ache 1994; Wachowiak et al. 1996).

Projection neurons (PNs) are the output pathways from the OL and AL and convey olfactory and multimodal information to the lateral protocerebrum. The somata of PNs receive their inputs from either the OL or AL and their axons ascend the olfactory globular tract (OGT) coursing within the eyestalks to target neurons in neuropils of the lateral protocerebrum, the medulla terminalis or the hemiellipsoid body, respectively (Mellon et al. 1992; Sullivan and Beltz 2001, 2005a) (Fig. 7.2b). The evolutionary history of the OL/AL/lateral protocerebrum axis exhibits extreme plasticity, confounding our current, imperfect understanding of the functional significance of its major and minor subdivisions. The hemiellipsoid body of *P. clarkii* is bilobed, and its principal local interneurons, the parasol cells, are anatomically and functionally separate in the two lobes. It has been suggested that common targeting of parasol cells in one lobe by bilateral AL inputs and of those in the other lobe by exclusively ipsilateral AL inputs could be the basis for comparing odorant signal strength at the two antennules (McKinzie et al. 2003). As the parasol cells of the *Procambarus* hemiellipsoid body receive their input primarily if not, exclusively, from the ALs, it should not be surprising that they are driven not only by exposure of the lateral antennular flagella to odors (Mellon and Alones 1997; Mellon and Wheeler 1999), but also by visual and tactile inputs (Mellon 2000). It can be speculated, therefore, that the hemiellipsoid bodies may be important in interpreting odors on the basis of environmental contexts, and in this respect they would be similar to the mushroom bodies in the insect brain that have a similar position within the central olfactory pathway.

7.7.2 Electrophysiology of Deutocerebral LNs and PNs

Electrophysiological studies of deutocerebral structures have been initiated in both crayfish and spiny lobster. Several types of LNs invest dendritic arbors within the OL and LAN of both crustaceans (Arbas et al. 1988; Mellon and Alones 1995; Schmidt and Ache 1996a, b). All of those observed so far are multiglomerular and respond to broad-spectrum complex odorants via their heavily arborized dendrites within the OL glomeruli (Fig. 7.8). In both species some of these LNs respond also to hydrodynamic stimulation of the antennules presumably via additional dendritic arbors that invest the LAN (Mellon and Alones 1995; Schmidt and Ache 1996a, b; Mellon 2005; Mellon and Humphrey 2007). Integration of odorant and

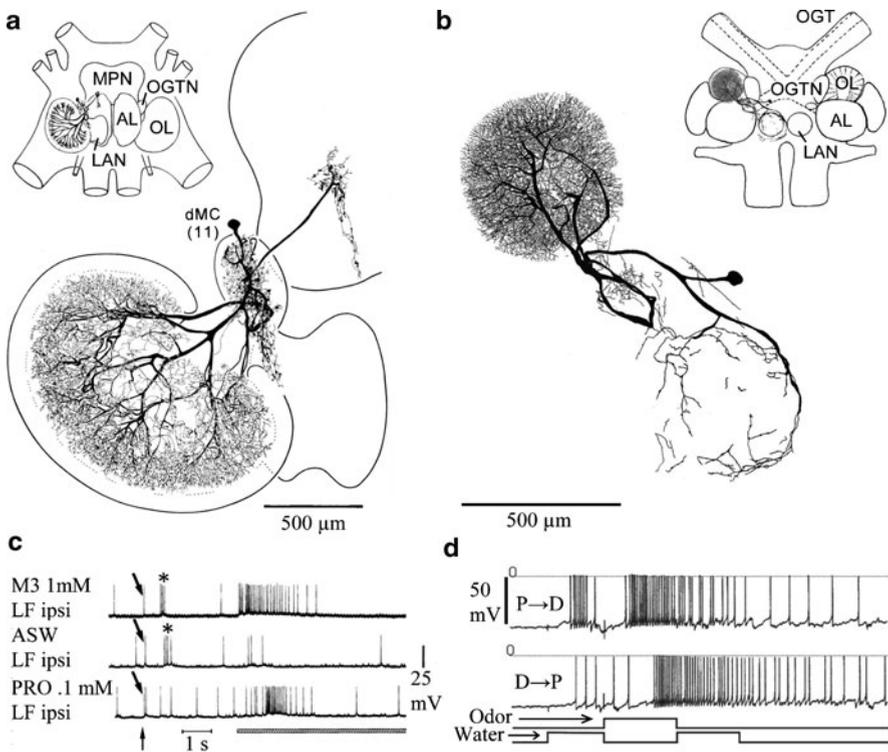


Fig. 7.8 Dendritic arborizations and electrical responses to odors of similar classes of multiglomerular local interneurons in the deutocerebrum of *P. argus* (a, c) and *P. clarkii* (b, d). Note that in both crustaceans the dendrites invest both the OL and LAN, and both respond not only to odorants but also to the onset of hydrodynamic flow past the antennular flagellum. (a, c) from Schmidt and Ache 1996b; (b) from Mellon and Alones 1995; (d) from Mellon and Humphrey 2007

hydrodynamic inputs is clearly a common property of the deutocerebrum. LNs are anatomically divided into those that enter the core of the OL and radiate their dendritic branches outwards through the glomeruli toward the cap and others that send their dendrites around the periphery of the OL and penetrate the glomerular individual columns through the cap region (Mellon and Alones 1995; Schmidt and Ache 1996b) (Fig. 7.2b).

Electrophysiological studies of OL PNs in both spiny lobster and crayfish suggest that the individual neurons receive their inputs from several different glomeruli (up to 80% of the available glomeruli in the spiny lobster) and thus the majority if not all of these PNs must be driven by a variety of odorant inputs (Wachowiak and Ache 1994; Schmidt and Ache 1996b; Sandeman and Mellon 2002). In a few successful penetrations with glass microelectrodes within the crayfish OL, electrical responses of PNs were obtained to stimulation of the ipsilateral antennule with single amino acids and simple mixtures (Mellon unpublished observations). The responses to odorant stimulation consisted of an initial brief excitation followed by a much more prolonged inhibition (Fig. 7.9). In *P. argus*, stimulation of the antennular nerve by single electrical shocks generated similarly patterned responses in individual PNs (Wachowiak and Ache 1994, 1998). Assuming that ORN input directly excites PNs and that the subsequent inhibition is secondary, a model for possible OL network properties can be proposed (Fig. 7.10). In this scheme individual ORNs send their axon terminals to a specific glomerulus dependent upon the olfactory determinant sensed by their dendritic receptor proteins. Several different PNs, on the other hand, may supply dendrites to any specific glomerulus; while some of these may receive strong excitatory ORN inputs in any one glomerulus, other PN dendrites within the same glomerulus may be excited less strongly but may themselves be more activated by ORNs in a different or several other glomeruli. Thus, no matter what the nature of the odorant (assumed to be a complex mix of olfactory determinants), all PNs will receive global inhibition following the selective excitation of only a few. Not included in the model is the evidence for widespread presynaptic inhibition of ORN terminals within the OL (Wachowiak and Ache 1998), possibly mediated by LNs whose dendrites course around the rim (glomerular cap region) of the OL, and presumably having a similar functional significance to the global postsynaptic inhibition as the one proposed in Fig. 7.10. This scheme predicts that individual ORNs and their target glomeruli represent individual olfactory determinants, while individual PNs are excited by mixtures of odor determinants, dependent upon the combination of glomeruli from which they receive strong inputs. The secondary inhibition provides an assurance that only the strongest ORN-PN input pathways are expressed as PN output spikes. The network would permit transient excitation of large numbers of different combinations of PNs, while suppressing other possible combinations, thereby forming a computational basis for identification of novel or previously learned combinations of olfactory determinants (“odors”) sensed by the ORN array.

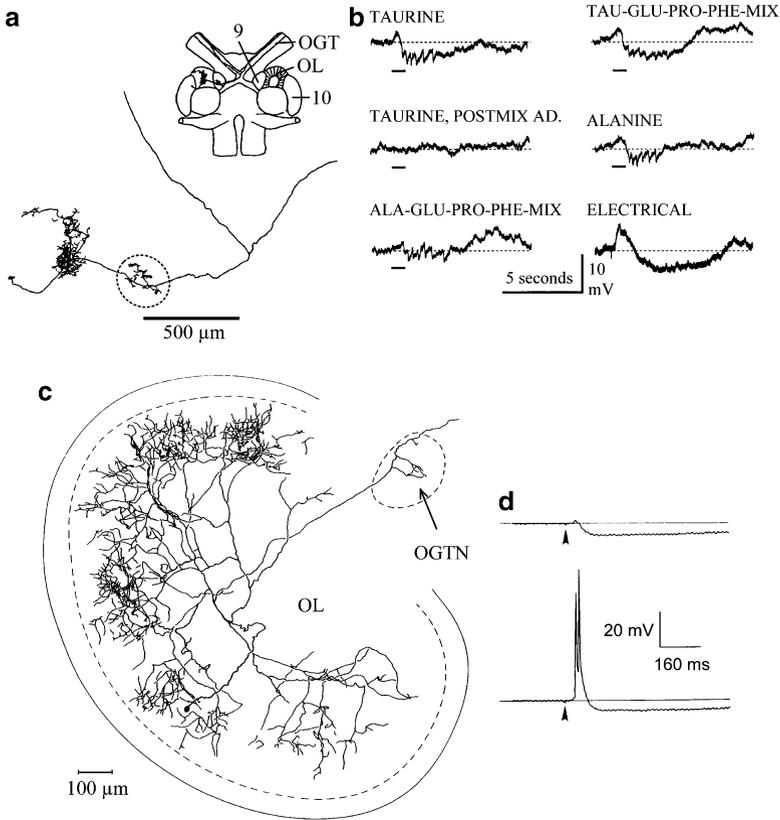


Fig. 7.9 Dendritic distribution and electrical responses of OL projection neurons (PNs) in *P. clarkii* (a, b) and *P. argus* (c, d). In both crustaceans the PNs are multiglomerular and respond with characteristic electrical profiles to broad-spectrum chemical stimulation (b) or to electrical stimulation (d). An initial excitatory phase is truncated by secondary inhibition which in *P. clarkii* does not persist. (a, b) from Sandeman and Mellon 2002; (c, d) from Wachowiak and Ache 1994

7.8 Persistent Neurogenesis in the Deutocerebrum

Beginning in 1997, using the mitotic incorporation of the thymidine analog 5-bromo-2'-deoxyuridine (BrdU), reports began to emerge of persistent neurogenesis in the brains of adult crustaceans (Schmidt 2007, and references therein; Sullivan et al. 2007, and references therein). In crayfish, spiny lobsters, and clawed lobsters persistent neurogenesis is a feature of PNs in cell cluster 10 and LNs in cell cluster 9 of the deutocerebrum. In the crabs *Cancer pagurus* and *Carcinus maenas*, (both brachyurans having highly reduced ALs), and in the shrimp *Sicyonia brevirostris* (having no ALs), neurogenesis was observed in adult animals in cell cluster

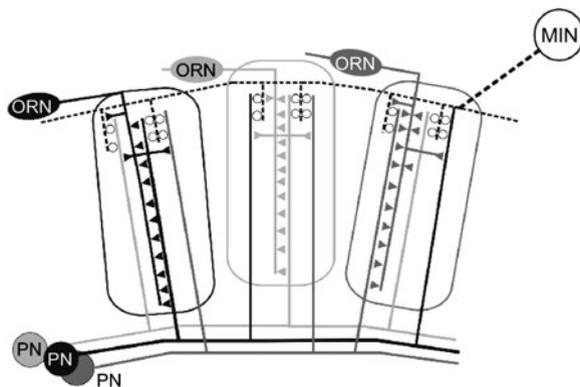


Fig. 7.10 Conceptual model to explain response profiles of crustacean OL PNs. Different classes of ORNs are tuned to specific olfactory determinants and each class terminates within a different glomerulus. OL PNs are less specific and present dendritic arbors across large numbers of glomeruli; however, it is hypothesized that each glomerulus is supplied by a preferred class of PN (same shade in the diagram) as well as a few other classes, so that the preferred PN receives most of the excitatory synaptic input from the ORNs in that glomerulus. In each glomerulus, the ORN input makes excitatory connections not only with PNs but also with multi-glomerular inhibitory neurons (MINs) that cause feedback inhibition to the intraglomerular PNs and feedforward inhibitory input to all other PNs. Output of the PNs is thus dependent upon matching their preferences with that of the array of glomeruli that they innervate. This scheme omits the evidence for presynaptic inhibition of individual ORN terminals within the OL, the functional significance of which could be similar to that of the MINs

10. In the crayfish *Cherax destructor*, convincing evidence has been obtained from PNs double labeled for BrdU and dextran that the nascent cells are incorporated into neuronal circuits of the OL and the AL (Sullivan and Beltz 2005b). In *Cancer pagurus*, *Carcinus maenas*, and the hermit crab *Pagurus bernhardus*, an additional site of neurogenesis was found in a cell cluster close to the hemiellipsoid body in the lateral protocerebrum (Schmidt 2007). In the brachyuran crab *Libinia emarginata*, adult neurogenesis has been found in cell clusters 10 and 9 and also near the hemiellipsoid body (Sullivan et al. 2007). This last finding is of particular interest because *L. emarginata* has a terminal adult molt, after which no new ORNs are incorporated into the antennule; nonetheless, neurogenesis in the brain continues throughout the animal's life, arguing against a role for nascent PNs as accommodation to the continuous addition of new ORNs. It would appear, therefore, that neuronal proliferation in cluster 10 is a general feature found in all decapods, whereas proliferation in cluster 9 and in a soma cluster close to the hemiellipsoid body only occurs in some species.

Why is persistent neurogenesis seen primarily in the central olfactory pathway of adult crustaceans, and what factors are involved in its control? Most, but not all crustaceans continue to grow throughout their lives which, in some cases, span decades. During this time, new sensilla of all types, including aesthetascs, are added to the antennules and other appendages and, as a result, new sensory neurons must

be incorporated within the framework of the OLs and other CNS regions. It has been argued (Schmidt 2007) that unlike the addition of somatotopic inputs to the structured brain neuropils of the deuto- and tritocerebrum, where simple branching of intrinsic neurons can accommodate new inputs in an orderly fashion, in the olfactory system novel combinations of ORN inputs that may be critical for the animals' survival can better be accommodated by additional classes of PNs that selectively provide an output pathway from those novel combinations of glomeruli that cannot be predicted. New PNs thus would form the substrate by which novel odors could be "learned" throughout the animals' lifetime. Although the number of classes of different ORNs may remain constant and the specific kinds of olfactory determinants the ORN array can detect is fixed throughout an animal's lifetime, new combinations of these determinants, i.e., complex environmental odors, will be interpreted through brain rewiring.

Several factors have been identified that impact persistent neurogenesis in the brains of decapod crustaceans. Environmental richness has been shown to influence not only proliferation in clusters 9 and 10 of the crayfish *C. destructor*, but also neuronal survival (Sandeman and Sandeman 2000). Young animals were confined either communally or individually, exposed to the same water conditions and maintained on the same feeding regimen. BrdU staining of nascent brain neurons indicated that brains of animals that had lived under "enriched conditions" had significantly more BrdU+ neurons than the brains of animals kept for the same amount of time in the "impoverished condition." The data suggest, therefore, that social interactions may play a critical role in regulating neurogenesis, at least in juvenile animals. Other influences on persistent neurogenesis in crustaceans have also been examined, including a possible role of sensory afferents (Schmidt 2007), circadian time (Goergen et al. 2002), and dietary omega-3 fatty acids (Beltz et al. 2007). The role of adult neurogenesis in the crustacean olfactory pathway is a relatively young field of study, but its implications are sufficiently profound that it will occupy an increasingly prominent place in the olfactory literature for the foreseeable future.

7.9 Summary and Outlook: What Can We Learn by Studying Crustacean Chemoreception?

In spite of more than 100 years of research, the physiology of crustacean chemoreception is far from being understood in detail. Several factors have delayed progress:

1. The aesthetascs are olfactory sensilla and usually all chemoreception mediated by the lateral antennular flagella (or the entire antennules) was attributed to them and hence considered as "olfaction." This interpretation is flawed because other chemosensory sensilla are associated with the aesthetascs and likely the activity of dCRNs in these bimodal sensilla was analyzed in most axon recordings from the lateral flagella.

2. Numerous attempts have been made to divide crustacean chemoreception in “olfaction” and “taste” along the lines of the perceived duplicity of chemical senses in mammals. These attempts have not been successful and in the meantime, the conceptual basis for grouping crustacean chemoreception into two chemical senses has become obsolete because the mammalian nose turned out to be a compound sense organ housing several chemosensory systems with distinct physiology and function (Munger et al. 2009).
3. The analysis of chemoreception in crustaceans has been focused almost exclusively on food-related chemicals. However, chemoreception is an important sensory channel in many other behavioral contexts such as social interactions and the assessment of danger from predation. Very likely different chemicals are important in these other contexts, but only recently progress was made in the identification of such chemicals and the sensilla detecting them.
4. The available data sets about the physiological properties of dCRNs and ORNs and about the functional organization of chemosensory pathways in the CNS are incongruent. While much more is known about the physiology of dCRNs of bimodal sensilla than of aesthetasc ORNs, the olfactory pathway in the brain is far better analyzed than any pathway receiving chemosensory input from bimodal sensilla.

Nevertheless, the following conclusion of broader significance emerges from research on crustacean chemoreception: Chemoreception in crustaceans is organized into two “modes,” for which we propose the terms “Olfaction” and “Distributed Chemoreception” (Fig. 7.1). This distinction is based on the profound difference in the construction of the two classes of crustacean chemosensory sensilla (aesthetascs vs. bimodal sensilla) and the equally profound difference in the neuroanatomical organization of the neuropils receiving their respective input (OL vs. sensory-motor neuropils). Since the aesthetasc-OL pathway shows obvious parallels to the olfactory pathway of vertebrates and insects (structure of receptor neurons, first synaptic relay with glomerular organization and adult neurogenesis), it seems justified to continue using the term “Olfaction” for chemoreception mediated by this pathway. The second mode, “Distributed Chemoreception,” however, cannot simply be equated with “taste” or with insect “contact chemoreception” which it parallels in terms of sensilla construction and functional neuroanatomy. “Distributed Chemoreception” comprises not only food localization and control of ingestion by bimodal sensilla on legs and mouthparts – i.e., taste – but also other bimodal sensilla on diverse appendages mediating different behaviors such as food search, grooming, or mating. The low threshold sensitivity of many dCRNs indicates that chemicals from distant sources can be detected (e.g., Thompson and Ache 1980; Derby and Atema 1982a) and the MRNs of bimodal sensilla are not exclusively tactile but in some cases are more sensitive and respond to water vibrations or flow (e.g., Derby 1982; Schmidt and Ache 1996a). Thus dCRNs and MRNs apparently not always require contact with a stimulus source but can be activated by distant stimulus sources as well.

To understand why in the marine environment two distinct modes of chemoreception evolved in crustaceans, we have to delineate what the unique properties of them are. As has been detailed above, “Olfaction” and Distributed Chemoreception” differ in: (A) the number of “chemosensory channels” (aesthetascs house several hundred ORNs, whereas bimodal sensilla typically contain fewer than 10 dCRNs), (B) the packaging of dCRNs with MRNs and the “topographic logic” information processing in the target neuropils (ORNs are not packaged with MRNs, their projections are nontopographic, and information processing in the OL is likely based on an odotopic map rather than a somatotopic map; Schmidt, 2007), (C) the integration with motor control (the target neuropils of dCRNs but not the OL contain motoneurons innervating the appendage providing the chemosensory input), and (D) adult neurogenesis in the target neuropils (in the OL but not in the target neuropils of bimodal sensilla, new interneurons are continuously generated in adults, although the sensory input from both aesthetascs and bimodal sensilla increases with each molt). Together these points suggest that the essence of “Olfaction” is to provide a detailed representation of the complex chemical environment integrating chemical signals from a variety of potentially interesting sources (food, conspecifics, predators, symbiotic partners, landscape) without reference to the location of stimuli and not to generate directed behavioral output by exerting motor control. In contrast, the essence of “Distributed Chemoreception” is to form representations of only few key chemicals (food-related chemicals, pheromones) within a somatotopic context provided by mechanoreception. The integration of chemo- and mechanosensory information permits pinpointing the location of chemical stimuli and can be utilized to control movements of the stimulated appendage via direct sensory-motor coupling and to orchestrate this with movements of other appendages.

As the functional organization of chemoreception in fish and crustaceans clearly demonstrates, the differentiation of chemoreception into distinct chemical senses must have its evolutionary origin in the aquatic environment. In light of this scenario, many apparently distinguishing features of taste and olfaction in terrestrial vertebrates and insects appear to be adaptations to the terrestrial environment, raising the question about the ultimate causes for the differentiation of chemoreception into different “modes.” This fundamental question can only be answered by further studying the functional organization and behavioral function of chemoreception in aquatic animals. We hope that doing so in crustaceans will continue to provide crucial insights into the evolutionary history of olfaction, taste, and other chemical senses.

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