Ultrastructure and Physiology of the Hooded Sensillum, a Bimodal Chemo-Mechanosensillum of Lobsters

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ABSTRACT

The antennules of decapod crustaceans are covered with thousands of chemosensilla that mediate odor discrimination and orientation behaviors. Most studies on chemoreception in decapods have focused on the prominent aesthetasc sensilla. However, previous behavioral studies on lobsters following selective sensillar ablation have revealed that input from nonaesthetasc antennular chemosensilla is sufficient for many odor-mediated behaviors. Our earlier examination of the setal types on the antennules of the Caribbean spiny lobster Panulirus argus revealed three types of nonaesthetasc chemosensilla. The most abundant and widely distributed of these is the hooded sensillum. The present study describes the detailed ultrastructure of antennular hooded sensilla and the physiological response properties of their receptor neurons. Light and scanning and transmission electron microscopy were used to examine structural characteristics, and electrophysiology was used to examine single-unit responses elicited by focal chemical and mechanical stimulation of antennular hooded sensilla. Hooded sensilla have a porous cuticle and are innervated by 9–10 chemosensory and 3 mechanosensory neurons whose dendrites project to the distal end of the sensillum. Hooded sensillar chemosensory neurons responded to waterborne chemicals, were responsive to only one of the six tested single compounds, and had different specificities. Hooded sensillar mechanosensory neurons were not spontaneously active. They had low sensitivity in that they responded to tactile but not waterborne vibrations, and they responded to sensillar deflection with phasic bursts of activity. These results support the idea that hooded sensilla are bimodal chemo-mechanosensilla and are receptors in an antennular chemosensory pathway that parallels the well-described aesthetasc chemosensory pathway.

Crustaceans rely on their chemical senses for many important behaviors. Chemoreception is used to detect food (Reeder and Ache, 1980; Zimmer-Faust et al., 1996; Giri and Dunham, 1999), to find mates (Gleeson, 1991; Giri and Dunham, 2000), to locate shelter (Ratchford and Eggleston, 1998, 2000), to avoid predators (Hazlett and Schoolmaster, 1998), and in social interactions among conspecifics (Caldwell and Dingle, 1985; Karavanich and Atema, 1998a,b; Zulandt Schneider and Moore, 1999, 2000). Here we present data on the structure and function of antennular sensilla that are candidate receptors for an antennular pathway in lobsters that mediates behaviors associated with the detection and discrimination of food odors.

The first antennae, or antennules, of decapod crustaceans are major chemosensory organs of these animals. The two most abundant chemoreceptor sensilla on the antennules are aesthetasc and hooded sensilla (Cate and Derby, 2001). Aesthetascs are the most thoroughly studied antennular chemoreceptor sensilla (ultrastructure: Laverack and Ardill, 1965; Spencer and Linberg, 1986;...
Grüntert and Ache, 1988; physiology: Spencer, 1986; Michel et al., 1991, 1993; Ache and Zhainazarov, 1995). They are located on the distal end of lateral flagellum of the antennules (Fig. 1). They are unimodal, each being innervated by ca. 300 chemoreceptor neurons (Grüntert and Ache, 1988; Steullet et al., 2000a) whose axons project to the olfactory lobes (Sandeman and Luff, 1973; Mellon and Munger, 1990). Hooded sensilla, on the other hand, have only recently been described (Cate and Derby, 2001). Ultrastructural evidence suggests that antennular hooded sensilla are bimodal (chemo-mechanosensilla; Cate and Derby, 2001), and the axons of their sensory neurons are thought to project to the lateral antennular neuropils and median antennular neuropil (Schmidt et al., 1992; Schmidt and Ache, 1992, 1996a,b). Hooded sensilla are located along the lateral and medial flagella of the antennules of many lobster species (Cate and Derby, 2000). They are also found on most body regions of the Caribbean spiny lobster Panulirus argus (Cate and Derby, 2000).

Behavioral studies on P. argus suggest that nonaesthetasc antennular sensilla are sufficient for many odor-mediated behaviors (Horner et al., 2000; Steullet et al., 2000b, 2001; Derby et al., 2001), even though antennular-mediated chemosensory behaviors are often attributed to reception solely by aesthetascs. Selective removal of either aesthetascs or nonaesthetascs does not significantly affect olfactory-mediated behaviors such as discrimination of, orientation to, and localization of food odors (Horner et al., 2000; Steullet et al., 2000b, 2002; Derby et al., 2001). Hooded sensilla are the most abundant class of nonaesthetasc sensilla (Cate and Derby, 2001) and are thus candidates for mediating these behaviors through a parallel antennular chemosensory pathway.

The results of this paper demonstrate that hooded sensilla have structural and functional properties of chemomechanosensilla. Hooded sensilla are innervated by dendrites of chemosensory neurons and mechanosensory neurons. We show that hooded sensillar chemosensory neurons respond to waterborne chemicals and have different response specificities. Furthermore, mechanosensory neurons that innervate hooded sensilla respond to sensillar deflection with phasic bursts of activity. Possible contributions of hooded sensilla to antennular chemoreception are discussed.

MATERIALS AND METHODS

Animals

All experiments were performed on young adult Caribbean spiny lobsters (Panulirus argus) of 60- to 80-mm carapace length. Animals were collected in the Florida Keys and held at Georgia State University. Lobsters were housed in 800-l aquaria at 20–25°C containing recirculating, filtered artificial seawater (Instant Ocean, Aquarium Systems, Mentor, OH) and fed shrimp and squid 3 times per week.

Light and electron microscopy

Lateral and medial flagella of the antennules were removed from lobsters 4–12 hours post-molt. Post-molt tissue was used because its cuticle was soft, undamaged, and clean of debris. The tissue was cut into small pieces (ca. 6 annuli), fixed overnight in 2.5% glutaraldehyde/1% paraformaldehyde in 0.2 M phosphate buffer (pH 7.4), post-fixed for 2 hours in 1% osmium tetroxide in 0.2 M phosphate buffer, dehydrated in a graded ethanol series, transferred to acetone, and infiltrated with Eponate 12 resin (Pelco, Redding, CA). Semithin sections of many lobsters were collected on 12-well slides (Erie Scientific, Portsmouth, NH), stained with toluidine blue, and examined by using a compound light microscope (Zeiss Axioskop, Carl Zeiss, Inc., Thornwood, NY). Thin sections were collected on uncoated nickel-mesh grids, stained with uranyl acetate and lead citrate, and viewed on a JEOL JEM-100 CX II transmission electron microscope operating at an accelerating voltage of 80 kV. Plate negatives were developed by using Kodak developing products (Eastman Kodak Co., Rochester, NY), scanned by using a Hewlett-Packard scanner (Hewlett-Packard, Co., Corvallis, OR), and captured by using Adobe Photoshop software (Adobe Systems, Inc., Mountain View, CA).

Crystal violet staining

The crystal violet technique of Slifer (1960) was used to examine the permeability of hooded sensilla on the antennular flagella. Antennules were fixed in 10% formalin in 0.2 M phosphate buffer (pH 7.4) for 24 hours, rinsed in water, and exposed to 0.5% crystal violet (anhydrous molecular weight = 408 daltons) in distilled water for 5–30 seconds. Antennules were rinsed in distilled water, dried under a lamp, cleared in xylene, and then viewed under a compound light microscope (Zeiss Axioskop). Images were digitally captured by using a Zeiss camera (Carl Zeiss, Inc) and Optronics acquisition software (Optronics, Inc., Goleta, CA). For all figures with photomicrographs (Figs. 1,3,4,9), digital images were placed and labeled by using Adobe Illustrator software (Adobe Systems, Inc.).

Electrophysiology

Removal of aesthetasc sensilla. Aesthetasc sensilla were removed from the lateral flagella by shaving 3–7 days before electrophysiology. The shaving protocol of Steullet et al. (2000b, 2001) was used to selectively remove aesthetasc, guard, and asymmetric setae without damaging other setae on the antennules (see Fig. 1C). Briefly, lobsters were restrained in a rectangular tub filled with artificial seawater (Instant Ocean™) and positioned under a dissecting microscope. A steel razor blade (0.2-mm wide) was used to cut aesthetasc, guard, and asymmetric setae at their bases. The entire procedure took approximately 20 minutes, and then the animal was marked and returned to its aquarium.

Recording set-up and data analysis. The lateral flagellum was removed from the animal 3–7 days after being shaved, and the distal end of the flagellum was placed into a teflon tube (length = 55 mm; inner diameter = 2 mm)
through which artificial sea water (ASW; Cavanaugh, 1964) was continuously flowing at a velocity of 3.5 cm/second (= 0.6 ml/second). At the proximal end of the flagellum, cuticle was removed to expose axons and the region of the flagellum. The artery was perfused with artificial lobster saline ([in mM]: 458 NaCl, 13.4 KCl, 13.6 CaCl₂, 9.8 MgCl₂, 14.1 Na₂SO₄, 3 HEPES, 1.9 glucose, 1.2 NaOH [pH adjusted to 7.4]) through a glass cannula at a flow rate of 2 ml/minute. Single unit activity was recorded extracellularly en passant from antennular axons by using a fine suction electrode (Derby, 1995). The suction recording electrode was connected to a differential AC amplifier (AM Systems, Everett, WA), an audio monitor, and a computer via an A/D board and Axoscope software (Axon Instruments, Burlingame, CA). Electrical responses were recorded by using the differential amplifier with the indifferent electrode effectively connected to ground. Analog signals were amplified and filtered below 60 Hz and above 10 kHz and then digitized and stored on a computer by using Axoscope software. Spike sorting and quantification of both instantaneous spike frequency and number of spikes elicited per response from the digitally stored traces were performed by using Datapac 2000 software (RUN Technologies, Mission Viejo, CA). Instantaneous frequency is based on the shortest interspike interval of the response. In two of the recordings from hooded sensillar chemosensory neurons, a mechanosensory neuron that was not associated with the hooded sensillum was also recorded (Figs. 6A, 7A). Datapac software was used to digitally remove this mechanosensory neuron before quantification of responses of the chemosensory neuron.

Either the chemical stimulation protocol (A, below) or the mechanical stimulation protocol (B, below) was followed for each antennule preparation.

A. Chemical stimulation protocol. Chemical stimuli were taурine, adenosine-5’-monophosphate (AMP), L-glutamate, glycine, L-proline, ammonium chloride, and artificial shrimp mixture (SM; an artificial mixture containing 29 components to a new location. If stimulation of more than one region was found, small regions (ca. 4 annuli) of the antennule were selectively stimulated to determine the location of the seta. If stimulation of more than one region evoked a response, then it was assumed that a proprioceptor was being stimulated, and the electrode was moved to a new location. Once a region was located, the pin probe was attached to a micromanipulator and used to selectively deflect each seta in the region. If we found a single seta that evoked a response, we then deflected all neighboring setae and the cuticle surrounding the seta to confirm. The seta was then presented with 10^{-4} M SM to test the mechanosensory neuron’s sensitivity to mechanical stimulation.

B. Mechanical stimulation protocol. For mechanical stimulation, the aesthetasc removal and recording procedures were the same, but the distal end of the flagellum was kept in an open chamber containing ASW. The search stimulus consisted of brushing the flagellum with a handheld paintbrush or a pin probe that consisted of a stainless steel pin (Minutien entomology pin, 0.1 mm diameter, Ianni Butterfly Enterprises, Cleveland, OH) glued to the end of a wooded stick using cyanoacrylate adhesive (Super Glue Corp., Rancho Cucamonga, CA). Once a responsive neuron was found, small regions (ca. 4 annuli) of the antennule were selectively stimulated to determine the location of the seta. If stimulation of more than one region evoked a response, then it was assumed that a proprioceptor was being stimulated, and the electrode was moved to a new location. Once a region was located, the pin probe was attached to a micromanipulator and used to selectively deflect each seta in the region. If we found a single seta that evoked a response, we then deflected all neighboring setae and the cuticle surrounding the seta to confirm. The seta was then presented with 10^{-4} M SM to test the mechanosensory neuron’s sensitivity to mechanical stimulation.

Confirmation of setal type. After the chemical or mechanical protocol was completed, the location of the seta was marked by scoring cuticle distal and proximal to the seta with a scalpel. The flagellum was cut one or two annuli distal to the responsive seta, and the seta was chemically (or mechanically) stimulated, to confirm that the response was not removed by the cut. Then the flagellum was cut one or two annuli proximal to the seta and the remainder of the antennule was stimulated, to confirm that stimulation of the remainder of the antennule did not evoke a response in the recorded axon. The piece of antennule with the marked seta was stored in 70% ethanol and later processed for scanning electron microscopy (as described above) to confirm the identity of the seta.

RESULTS
Distribution of hooded sensilla on the antennule
The lobster’s antennule has four segments (Fig. 1A). The most distal segment bifurcates into two flagella, lat-
eral and medial. Each flagellum has 500–600 hooded sensilla that are distributed on most annuli (segments) except for those on the distal tip (Cate and Derby, 2001). On medial flagella and on the proximal half of lateral flagella, hooded sensilla are present on all sides of the flagella (Fig. 1D). In the tuft region on the distal half of lateral flagella (Fig 1C), they are present on all sides except for the ventral surface (where the aesthetasc tuft is located). The number of annuli (Harrison et al., 2001), and thus the number of hooded sensilla, increases with the size of the animal.

Ultrastructure of hooded sensilla

Cuticular structures. Antennular hooded sensilla are ca. 55-μm-long and ca. 15-μm-wide at their base. They have a short, stout shaft that tapers distally and has serrations on one side at the distal end (Fig. 1B). Short setules project from the setal shaft just below and in front of the serrations on the distal end. Longer setules project from the shaft on the opposite side from the serrations. The longer setules project past the distal tip of the shaft and appear as a hood over the serrate end of the shaft (Fig. 1B).
Hooded sensilla articulate within shallow pits. There are no elaborate joint structures where the sensilla articulate with the cuticle of the flagellum. They insert in the proximal edge of the pits and project at an angle of ca. 20 degrees toward the distal end of the flagellum (Fig. 1C,D).

The porosity of the cuticle of hooded sensilla was examined by using the crystal violet technique (Slifer, 1960). Lateral and medial flagella from 4 antennules were used for this investigation. Within 5 seconds of exposure to crystal violet, the distal serrate region of hooded sensilla was darkly stained (Fig. 1E). This stain could not be removed by rinsing the cuticle. The proximal region of the setal shaft did not stain with crystal violet even after increasing the exposure time to 30 seconds. Thus, crystal violet staining (Fig. 1E) suggests that cuticle in the distal part of the sensillum is permeable to small, water-soluble molecules.

The ultrastructure of 5 hooded sensilla was examined. Sections through the distal serrate region of hooded sensilla (Fig. 2) revealed that the cuticle of the shaft has wall pores (Fig. 3A,B) and that the setal shaft has a lumen, or outer lymph cavity, that is ca. 1-μm-wide in the distal end and ca. 7 μm in diameter at the base (Fig. 3). Taken together, these results suggest that porous cuticle in the distal part of the sensillum may allow chemical stimuli from the external environment to enter the lymph cavity within the sensillum.

**Sensory cells.** The primary sensory cells that innervate hooded sensilla have outer and inner dendritic segments (Fig. 2). Inner dendritic segments extend from the inner receptor lymph cavity to the sensory somata, which are located 500–600 μm under the cuticle of the flagellum.

Hooded sensilla are innervated by 12 to 13 primary sensory neurons (n = 5). The outer dendritic segments are modified cilia. All dendrites are unbranched and project to the distal end of the setal shaft within the outer lymph cavity (Figs. 2, 3A,B). Microtubules are present in all outer dendritic segments (Figs. 3G, 4A). Within the lumen at the base of the sensillum, the outer dendritic segments are enclosed in an osmiophilic dendritic sheath (Fig. 3F,G). At the transitional region between the inner and outer dendritic segments, the inner lymph cavity measures 2.5–3.0 μm in diameter (Fig. 4B,C). In this transitional region, the innermost auxiliary cell contains a dense meshwork of osmiophilic filaments called the scolopale (Figs. 2, 4A–E). The ciliary regions, basal bodies, and ciliary rootlets of the dendrites are located within the transitional region (Figs. 2, 4B–E). Each dendrite has a ciliary segment with a 9 × 2 + 0 organization (Fig. 4B,C), a single basal body (Fig. 4B), and a well-developed ciliary rootlet within the distal region of the inner dendritic segment (Fig. 4E).

The sensory neurons innervating each hooded sensillum do not have obvious differences in the morphology of their inner dendritic segments or cell bodies. However, distinct differences in their outer dendritic segments and in the structures within the transitional region are apparent. These are thought to reflect differences in modalities of the sensory cells and are discussed below (see Modality-specific features of sensory cells).
Fig. 3. Transmission electron micrographs of ultrastructural features of antennular hooded sensilla. Locations of sections are indicated in Figure 2. A,B: Sections through the serrate region at the distal end of the sensillum. Note the outer lymph cavity (olc) within the sensillum and wall pores through the cuticle. C,D: Sections through the midshaft region of the sensillum. C: The lumen of the sensillum (asterisk) is in the center of the setal shaft, and setules attach on all sides of the setal shaft. D: Higher magnification of boxed area in C showing outer dendritic segments and auxiliary cell processes (arrowhead) within the lumen of the sensillum. E,F: Sections through the base of the setal shaft. E: Outer dendritic segments (arrow) are bundled within the lumen in the proximal setal shaft. F: Higher magnification of dendrite bundle in the base of the setal shaft. Thirteen outer dendritic segments containing microtubules (mt) are surrounded by a dendritic sheath (ds). Auxiliary cell processes (arrowhead) are present within the lumen. c, cuticle. Scale bars = 2 μm in A, 0.2 μm in B,F, 2.5 μm in C,E, 0.3 μm in D.
Fig. 4. Transmission electron micrographs of ultrastructural features of dendrites innervating hooded sensilla. Locations of sections are indicated in Figure 2. A: The distal end of the transitional region. In this region, the outer dendritic segments are surrounded by a dense meshwork of osmiophilic filaments called the scolopale. Three of the dendrites (arrows) have more densely packed microtubules (mt), a characteristic of mechanosensory dendrites (see text). B–D: The center of the transitional region. B: Three dendrites (arrows) within the inner lymph cavity (ilc) begin their ciliary regions more distal to the others. In this region, scolopale partially surrounds the lymph cavity. C: High-magnification view of two of the dendrites shown in B within the inner lymph cavity (ilc). Ciliary regions of these dendrites are of the 9 × 2 + 0 type and have dynein arm-like protuberances (arrows) on their microtubule doublets. D: Basal bodies of dendritic ciliary segments within the inner lymph cavity (ilc) in the transitional region. E: The proximal end of the transitional region. In three dendrites, rootlets of ciliary segments (arrows) are associated with the scolopale of the innermost auxiliary cell. The ciliary rootlets of other dendrites (asterisks) are not associated with scolopale. Auxiliary cell processes (arrowhead) surround the dendrites. F: Proximal to the transitional region. In this region, the 13 inner dendritic segments contain mitochondria (arrows) and have no obvious morphological differences. Scale bars = 0.2 μm in A,C,D, 0.5 μm in B,F, 1 μm in E.
**Auxiliary cells.** Processes of glial cells, called auxiliary cells, surround the dendritic segments of sensory cells innervating hooded sensilla. Auxiliary cells envelop the outer dendritic segments within the lumen of the setal shaft (Fig. 3C,D). More proximally, at the base of the setal shaft, auxiliary cell processes surround the dendritic sheath, which encircles the outer dendritic segments (Fig. 3E–G). Within the transitional region, auxiliary cells surround the inner lymph cavity and the innermost auxiliary cell contains the scolopale (defined above; Fig. 4A–C,E). Proximal to the inner lymph cavity, auxiliary cell processes surround the inner dendritic segments (Fig. 4E,F).

**Modality-specific features of sensory cells.** Modality-specific features for dendrites of chemosensory neurons and mechanosensory neurons have been identified in crustacean sensilla (Crouau, 1982; Altner et al., 1983, 1986; Gnatzy et al., 1984; Schmidt and Gnatzy, 1984; Spencer and Linberg, 1986; Grünert and Ache, 1988; Schmidt, 1989). The features described by these studies can be used to distinguish mechanosensory (type I) and chemosensory (type II) dendrites (Schmidt and Gnatzy, 1984; Schmidt, 1989). By using these features, we recently proposed that 9–10 chemosensory neurons and 3 mechanosensory neurons innervate hooded sensilla (Cate and Derby, 2001). Here, we support and extend these findings.

Modality-specific features identified in hooded sensillar dendrites are the following: (1) outer dendritic segments of mechanosensory neurons have many densely packed microtubules, whereas dendrites of chemosensory neurons have fewer and less densely packed microtubules (Fig. 4A); (2) mechanosensory dendrites are more closely associated with the scolopale than are chemosensory dendrites (Fig. 4B,C); (3) the ciliary segments of mechanosensory dendrites are located more proximal than those of chemosensory dendrites (Fig. 4B); (4) ciliary segments of mechanosensory dendrites have A-tubules with dynein arm-like protuberances (Fig. 4B,C), whereas chemosensory dendrites do not have these protuberances (not shown); and (5) ciliary rootlets of mechanosensory dendrites embed within the scolopale, whereas chemosensory ciliary rootlets are not associated with the scolopale (Fig. 4E). In addition, hooded sensillar dendrites with mechanosensory characteristics were always located at the outer side of the bundle of dendrites (nearest to the cuticle of the flagellum) similar to that observed for dendrites of mechanosensory neurons innervating fringed sensilla (Altner et al., 1983).

**Physiological properties of hooded sensilla**

We recorded from approximately 300 chemosensory neurons by using the antennular preparation in which aesthetasc, asymmetric, guard, and companion setae were removed. This suggests that the lateral flagellum still has a high chemoreceptive capacity in the absence of these sensilla, due to any of a number of other known or putative nonaesthetasc chemosensilla (Cate and Derby, 2001). These nonaesthetasc chemosensory neurons were responsive to adenosine-5'-monophosphate (AMP), L-proline, glycine, L-glutamate, taurine, and/or ammonium chloride (i.e., all of the single compounds tested). The specificity of the neurons differed in the number of excitatory compounds and the identity of the most excitatory compound. However, electrophysiological data were included in the following analysis only if the entire protocol was completed, including controls and ending with confirmation of the identity of the setal type by scanning electron microcopy (see Materials and Methods). The single-unit activity was recorded from 3 chemosensory neurons and 3 mechanosensory neurons that were identified with certainty to innervate hooded sensilla. Each of these 6 neurons was from a different lateral flagellum and a different animal. None of the 6 neurons was sensitive to both mechanical and chemical stimulation.

**Response sensitivity and specificity of single chemosensory neurons.** All 3 hooded sensillar chemosensory neurons had different response sensitivities and/or specificities. All were excited by the search stimulus, artificial SM, and did not respond to ASW (Figs. 5A, 6A, 7A). Each of the chemosensory neurons responded to only 1 of the 6 tested single compounds, and responses to these single compounds were excitatory. Two hooded sensillar chemosensory neurons responded only to taurine (HSC1 and HSC2) and 1 responded only to ammonium (HSC3). HSC1 and HSC2 did not show spontaneous activity, whereas HSC3 exhibited a low rate of spontaneous spiking (1.4 spikes/second; Fig. 7A). HSC1 had a threshold at or below 10\(^{-4}\) M for SM and taurine (Fig. 5B,C). HSC2 and HSC3 had relatively higher response thresholds. HSC2 had a threshold of 10\(^{-4}\) M for SM and 10\(^{-5}\) M for taurine (Fig. 6B,C). HSC3 had a threshold of 10\(^{-3}\) M for SM and at or below 10\(^{-5}\) M for ammonium (Fig. 7B,C).

**Concentration-response relationship for single chemosensory neurons.** The magnitude of the responses of hooded sensillar chemosensory neurons increased with increasing stimulus concentrations. For all 3 chemosensory neurons, the instantaneous spiking frequency increased with increasing stimulus concentration over a range of 10\(^{-5}\)–10\(^{-2}\) M SM and over the same concentration range for taurine (HSC1 and HSC2) or ammonium (HSC3; Fig. 8A). However, the number of spikes per response to SM increased over a more narrow range, 10\(^{-5}\)–10\(^{-4}\) M for HSC1 and HSC2, and 10\(^{-4}\)–10\(^{-3}\) M for HSC3 (Fig. 8B). For both taurine-sensitive cells, HSC1 and HSC2, the number of spikes per response increased over a range of 10\(^{-6}\)–10\(^{-4}\) M (Fig. 8B), even though the instantaneous spiking frequency increased over a higher range (Fig. 8A). Similarly, the instantaneous spiking frequency of HSC3 in response to ammonium increased from 10\(^{-4}\)–10\(^{-2}\) M, the number of spikes increased only up to 10\(^{-4}\) M and decreased at higher concentrations (Fig. 8B). This demonstrated rapid adaptation at high stimulus concentrations.

**Responses of single mechanosensory neurons.** The 3 hooded sensillar mechanosensory neurons did not have spontaneous spiking activity and did not respond to changes in flow rate of ASW superfusing the preparation. All 3 mechanosensory neurons responded to displacement from rest (by deflecting the sensillum toward the flagellum with a probe; Fig. 9A) with bursts of spikes that rapidly adapted after maximal deflection (Fig. 9B) and all followed repetitive stimulations with bursts of spiking (Fig. 9C). For the neurons, the duration of the response to a 1.5- to 2.0-second constant stimulation was 352.2 ± 16.8 milliseconds (mean ± S.E.M.; n = 6; or n = 2 for each of the 3 neurons). The maximum instantaneous frequency of the response to mechanical stimulation was 175.9 ± 9.8 spikes per second (mean ± S.E.M.; n = 29; or n = 9–10 for each neuron). All 3 neurons followed repetitive stimulations up to at least 2 deflections per second. Deflecting neighboring sensilla (Fig. 9D) or touching the cuticle surrounding the sensilla did not stimulate the neurons.
The results of this study indicate that hooded sensilla are bimodal, being innervated by both chemosensory neurons and mechanosensory neurons. The chemosensory neurons are responsive to waterborne chemicals and have different response specificities. The mechanosensory neurons respond to deflection with phasic bursts of activity that rapidly adapt. These results are consistent with the idea that hooded sensilla are capable of mediating olfactory behavior through an antennular chemosensory pathway that parallels the well-studied aesthetasc/olfactory lobe pathway, and incorporates both chemosensory and mechanosensory reception.

**Structure and function of hooded sensillar neurons**

Hooded sensilla meet the structural and functional requirements for receptors of an antennular chemosensory pathway. This pathway was proposed on the basis of behavioral studies that show that ablation of aesthetasc does not eliminate antennular chemoreception (Horner et al., 2000; Steullet et al., 2000b, 2001, 2002; Derby et al., 2001). Hooded sensilla are the most abundant and widely distributed nonaesthetasc sensilla (Cate and Derby, 2001) and are thus the main candidates for nonaesthetasc receptors of this pathway.

Structural features can be diagnostic of the modality of crustacean chemosensory neurons and mechanosensory neurons. Features of dendrites associated with hooded sensilla (Fig. 4) have been used here as an indication of modality, and physiological responses following focal stimulation of hooded sensilla (Figs. 5–7, 9) support these findings. Chemosensory neurons from various crustacean sensillar types share common morphological features in their dendrites (fringed setae: Altner et al., 1983; funnelcanal organs: Schmidt and Gnatzy, 1984, 1989; aesthetascs: Spencer, 1986; Spencer and Linberg, 1986; Grünert and Ache, 1988; Michel et al., 1991; Ache and Zhanazarov, 1995; hair peg organs: Schmidt, 1989; hooded sensilla: present study). Structural characteristics
of dendrites also reflect the modality of chemosensory neurons innervating insect sensilla (Altner and Prillinger, 1980; Lee and Strausfeld, 1990; Shanbhag et al., 1999). Similarly, common structural features of mechanosensory dendrites have also been identified in crustaceans (Mellon, 1963; Takahata and Hisada, 1979; Crouau, 1982; Koyama and Shimozawa, 1982; Altner et al., 1983; Schmidt and Gnatzy, 1984; Espeel, 1985; Schmidt, 1989; Hertwig et al., 1991; Cate and Roye, 1997) and insects (Thurm, 1964; Gaffal et al., 1975; Hansen, 1978; Altner and Prillinger, 1980; Barth, 1981). Thus, structural features can be good indicators of the modality of sensory neurons innervating arthropod sensilla.

One of the questions raised by this study was whether hooded sensilla are innervated by bimodal neurons. This does not appear to be the case. Although the sensilla are bimodal, this is a consequence of their innervation by two distinct populations of unimodal neurons, one population being chemosensory and the other mechanosensory. Bimodal (chemo- and mechanosensory) neurons do not appear to be common in crustaceans, having been described only once (Hatt, 1986). Our results suggest that two populations of unimodal neurons innervate hooded sensilla, because all hooded sensillar neurons that we examined fit within one of two types based on structural and electrophysiological characteristics.

A further question is how the sensitivities and specificities of chemosensory neurons that innervate nonaesthetascs (including hooded sensilla) and aesthetascs compare. Two separate studies examined the thresholds of chemosensory neurons in the antennules of the spiny lobster. Fuzessery (1978) found that populations of neurons from the aesthetasc-bearing region of the lateral flagellum and those from the nonaesthetasc-bearing regions of the lateral and medial flagella had similar sensitivity to taurine. In addition, Thompson and Ache (1980) reported that chemosensory neurons of the lateral and medial flagella do not differ with respect to threshold. Although these studies did not identify the specific sensillar types that the neurons were associated with, they suggest that chemical response thresholds are similar in aesthetasc and nonaesthetasc regions of the flagella.

The present study supports the idea that hooded sensillar chemosensory neurons have different specificities and

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Fig. 6. Electrophysiological responses of hooded sensillar chemoreceptor neuron 2 (HSC2). A: Raw traces of responses of HSC2 to artificial sea water (ASW) (1) and to 1 mM artificial shrimp mixture (2) presented to the antennular flagellum. The stimulus channel shows the opening and closing of the stimulation valve as described in Figure 5. The traces contain two units, the hooded sensillar chemoreceptor neuron (arrow) and a larger mechanoreceptive unit not associated with the hooded sensillum (double arrow). B: Responses of HSC2 to $10^{-5}$–$10^{-2}$ M artificial shrimp mixture. C: Responses of HSC2 to $10^{-5}$–$10^{-2}$ M taurine. The vertical bars in B and C represent the time course of stimulation as described in Figure 5.
respond to some of the same chemicals as do aesthetasc chemosensory neurons. Aesthetasc chemosensory neurons have differing specificities, as shown by patch clamp recordings (Michel et al., 1993; Simon and Derby, 1995). In the present study, recordings were made from approximately 300 chemosensory neurons in lateral flagella that had aesthetascs, guard, companion, and asymmetric setae removed. Three of these neurons were confirmed to be associated with hooded sensilla and responded to ammonium chloride or taurine. The other nonaesthetasc chemosensory neurons were responsive to AMP, L-proline, glycine, L-glutamate, taurine, and/or ammonium chloride. Hooded sensilla probably house some of these other nonaesthetasc chemosensory neurons as well as some of the chemosensory neurons in past recordings made from lateral flagella, because hooded sensilla constitute 29% of the sensilla on the lateral and medial flagella and 46% of the nonaesthetasc sensilla on the lateral flagellum (Cate and Derby, 2001).

**Dual antennular chemosensory pathways**

Several types of sensilla contribute to the chemosensory capacity of the antennules. Structural evidence suggests that there are at least four types of antennular chemosensilla: aesthetasc, hooded, long simple, and medium simple (Grünewalt and Ache, 1988; Cate and Derby, 2001). Physiological evidence presented in this and previous studies confirms that both hooded and aesthetasc sensilla respond to behaviorally relevant chemical cues (Spencer, 1986; Michel et al., 1991, 1993; Ache and Zhainazarov, 1995; present study). Hooded and aesthetasc sensilla are the two most abundant sensilla on the antennular flagella.

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**Fig. 7. Electrophysiological responses of hooded sensillar chemoreceptor neuron 3 (HSC3).**

A: Raw traces of responses of HSC3 to artificial sea water (ASW) (1) and to 1 mM artificial shrimp mixture (2) presented to the antennular flagellum. The stimulus channel shows the opening and closing of the stimulation valve as described in Figure 5. The traces contain two units, the hooded sensillar chemoreceptor neuron (arrow) and a larger mechanoreceptive unit not associated with the hooded sensillum (double arrow). B: Responses of HSC3 to $10^{-6}$–$10^{-2}$ M artificial shrimp mixture. C: Responses of HSC3 to $10^{-5}$–$10^{-2}$ M ammonium. The vertical bars in B and C represent the time course of stimulation as described in Figure 5.
It is well established that aesthetascs are involved in odor-mediated tasks (Reeder and Ache, 1980; Devine and Atema, 1982; Gleeson, 1991; Horner et al., 2000; Steullet et al., 2000, 2001, 2002; Derby et al., 2001). Recent studies have shown that nonaesthetascs are involved in odor-mediated tasks (Horner et al., 2000; Steullet et al., 2000b, 2001, 2002; Derby et al., 2001), supporting evidence that at least two chemosensory pathways from the antennules to the central nervous system exist (Schmidt et al., 1992; Schmidt and Ache, 1992, 1996a,b).

The two pathways are anatomically distinct, despite the fact that they have partially overlapping functions (Horner et al., 2000; Steullet et al., 2000b, 2001, 2002; Derby et al., 2001). In the first pathway, chemosensory neurons that innervate aesthetasc sensilla project to the glomerularly organized olfactory lobes (OL). It is also possible that a small number of additional afferents, possibly mechanosensory, project to the OLs (Schmidt and Ache, 1992, 1996a,b). In the second pathway, both chemosensory and mechanosensory neurons that innervate hooded sensilla project to the stratified lateral antennular neuropils (LAN) and possibly to the median antennular neuropil (MAN; Schmidt et al., 1992; Schmidt and Ache, 1996a,b). Another functional distinction between the two pathways may be in pheromone reception. The possibility that aesthetasc and nonaesthetasc pathways differ in responsiveness to pheromones has only been examined in detail in blue crabs (Gleeson, 1991). In these animals, aesthetascs are responsible for pheromone reception and are considerably more numerous than nonaesthetasc antennular sensilla (Gleeson, 1982). In spiny lobsters, it is not known whether the aesthetasc

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**Fig. 8.** Concentration-response plots for hooded sensillum chemoreceptors (HSCs). **A:** Plots of instantaneous spiking frequency for HSC1 (1), HSC2 (2), and HSC3 (3). Instantaneous frequency is based on the shortest interspike interval of the response. **B:** Plots of total number of spikes per response for HSC1 (1), HSC2 (2), and HSC3 (3).
pathway mediates responses to social pheromones (Ratchford and Eggleston, 1998, 2000) or sex pheromones. Another possible distinction between the pathways is that the nonaesthetasc/LAN-MAN pathway, because of the large amount of input from bimodal sensilla, may allow integration of input from chemosensory and mechanosensory neurons at the same spatial location along the antennule. This is explored more in the next section.

Hooded sensilla packaging and central projections

It remains to be determined whether there is any functional significance of packaging both chemosensory neurons and mechanosensory neurons into hooded sensilla. Hooded sensillar chemosensory neurons respond to waterborne chemicals (Figs. 5–7) independent of mechanical stimulation. Thus, the coexistence of neurons might be related to packaging restrictions rather than some functional requirement. Nevertheless, the possibility remains that this system is wired to detect coincidence of chemical and mechanical stimulation. For example, if the chemosensory and mechanosensory neurons project together to the central nervous system in a topotopic arrangement, this might permit the spatial location of chemical stimuli, mechanical stimuli, or coincident chemical and mechanical stimuli. In this way, hooded sensilla could function not only by responding to waterborne chemicals to mediate olfactory behavior, but also by responding to chemical and/or tactile stimuli to mediate reflexive movements of the antennules. Consistent with this, there is long-standing evidence that the LAN and MAN are centers for antennular sensory-motor integration and are responsible for driving antennular movements (Maynard, 1966; Schmidt and Ache, 1996a). Topotopic projections from bimodal sensilla are found in locusts, where both chemosensory neurons and mechanosensory neurons of bimodal sensilla on the legs project together into thoracic ganglia (Newland et al., 2000). These sensilla are considered contact chemoreceptors for gustatory processing and are likely to function in local reflex movements in response to either chemical or mechanical stimulation (Rogers and Newland, 2000).

In contrast, the central projections of chemosensory neurons and mechanosensory neurons from hooded sensilla might be separate and might not preserve the spatial location of stimuli. In the blowfly labella (i.e., mouthparts; Yetman and Pollack, 1986; Edgecomb and Murdoch, 1992) and in the legs of the blowfly and fruitfly (Murphey et al., 1989), projections from mechanosensory and chemosensory neurons of contact chemoreceptors segregate. In the blowfly labella, mechanosensory neurons project somatotopically, whereas the chemosensory neurons project to a
separate location in the neuropil. In this case, the functional significance of colocalization of chemosensory neurons and mechanosensory neurons into bimodal sensilla is not obvious and may simply be a reflection of efficient packaging. In the case of the lobster antennule, even if the projections from hooded sensillar neurons into the LAN/MAN are not topotopic, excitation of hooded sensilla mechanosensory neurons, by flicking the antennules or by touch, might interact with chemosensory input by sensitizing the circuits for chemosensory input. Further investigation of the central projections from individual hooded sensillar neurons is needed to determine whether positional information from mechanosensory neurons and/or chemosensory neurons is preserved in the central nervous system.

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