COMMENTARY

Smelling, feeling, tasting and touching: behavioral and neural integration of antennular chemosensory and mechanosensory inputs in the crayfish

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Summary

Crustaceans possess two pairs of prominent, movable sense organs on the rostral aspect of their bodies termed antennae: (1) a relatively short, usually bifurcate pair, the 1st antennae, also referred to as antennules, and (2) a much longer, uniramous pair, the 2nd antennae, or just 'antennae'. The antennules are equipped with diverse arrays of six or more types of cuticular setae, most of which are believed to have a sensory function. Axons from these structures course within the antennular nerve to the deutocerebrum, a large middle brain region that is known to receive chemoreceptor and mechanoreceptor inputs. In crayfish, axons from two kinds of single sensory-function setae, the olfactory receptor aesthetasc sensilla and as yet unidentified hydrodynamic sensilla, on the lateral antennular flagellum terminate, respectively, within the ipsilateral olfactory lobe and the lateral antennular neuropil of the deutocerebrum, where their activity generates synaptic potentials in local interneurons having dendritic fields that span both of those regions. It has been suggested that the short-latency hydrodynamic input gates or otherwise supplements the olfactory input signals. Much less is known about the functional capabilities of the other sensillar types on the antennular flagella, including the bimodal sensilla: how their inputs are distributed to the various neuropils of the deutocerebrum, whether they target common or separate brain neurons, and the nature, if any, of their functional relationships to the aesthetasc and hydrodynamic sensilla. Integrated processing of chemical and hydrodynamic signals undoubtedly plays an important role in locating odorant sources, perhaps by detecting boundaries of odorant plumes (tropotactic discrimination); other less-plausible strategies include time averaging of turbulent odorant signals and determination of concentration slopes within turbulence-generated odorant patches. These gaps in our understanding present important, but surmountable, experimental challenges for the future.

Key words: Crustacea, mechanoreception, olfaction.

Introduction

With the notable exception of the highly developed visual capacity in stomatopods and some semi-terrestrial grapsoid crabs, the survival of most aquatic crustaceans depends heavily upon information gleaned from the surrounding fluid medium: its mass motion, the turbulent eddies imbedded within it, and its cargo of dissolved chemical agents (Weissburg, 2000; Webster and Weissburg, 2001; Atema, 1995; Atema, 1996; Grasso and Basil, 2002). Although most large crustaceans and many smaller ones are benthic dwellers and feed upon small invertebrates and detritus in the substrate, they depend upon the movement of the fluid medium for information about distant food sources, conspecific sex pheromones and environmental properties, and for evidence of nearby predators. This task is complicated by the turbulence generated within a transition zone above the substrate (Weissburg, 2000) that transforms laminar advective fluid movement at the substrate into chaotic eddies that may extend a meter or more above the substrate. The size of most macrocrustaceans suggests that they will encounter turbulence at least within the transition zone, if not above it; it is therefore of crucial importance that bulk fluid flow as well as the presence of local eddies be apprehended. How do the collective unimodal chemosensitive and hydrodynamically sensitive setae together with the bimodal chemo-mechanosensitive setae accomplish the important task of detecting critically salient

chemical sources within this turbulent environment? Near-field hydrodynamic receptor sensilla that detect fluid movement are present over a large proportion of the decapod body surface, including the cephalothorax (Mellon, 1963), the tailfan (Wiese, 1976; Douglass and Wilkens, 1998), the first antennae, or antennules, (Mellon and Christisen-Lagay, 2008; Mellon, 2010; Mellon and Abdul Hamid, 2012) and the 2nd antennae (Wilkens et al., 1996). Sensors subserving distributed chemosensitivity (Schmidt and Mellon, 2010), usually occurring as bimodal chemo-mechanosensitive sensilla (Mellon, 2007), occur on the pereiopods, including the claws, the maxillae, maxillipeds and mandibles, the antennules and probably on the second antennae as well (Altner et al., 1983; Derby and Atema, 1982; Derby, 1989). Although several studies have elucidated the functional capabilities of the statocysts, the organs of balance and angular acceleration on the basal segment of the antennules in all decapod crustaceans (Cohen, 1955; Sandeman and Okajima, 1972), in this Commentary I will confine my discussion to the integration of chemical and mechanical inputs from sensilla found specifically on the antennular flagella, primarily from studies on a few decapod crustaceans. Many questions remain concerning the broad variability in morphology and distribution patterns of the various antennular sensilla and their central targets, even as recent studies have begun to elucidate their functional attributes. Examination of the integration of chemical
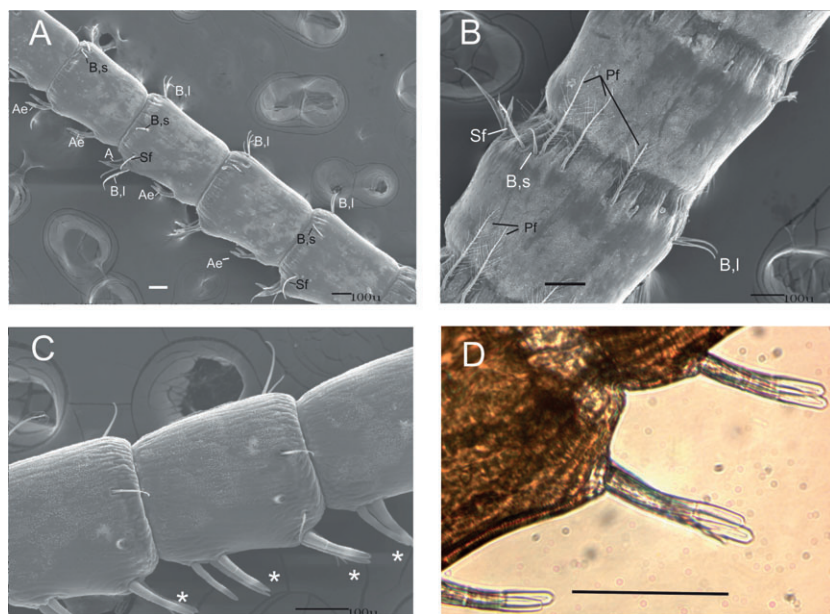
Fig. 1. Scanning electron micrographs of a lateral antennular flagellum of *Procambarus clarkii*, showing different types of setae. (A) A view of the distal lateral flagellum bearing aesthetasc sensilla (Ae), large beaked sensilla (B,l), small beaked sensilla (B,s), an asymmetric seta (A) and standing feathered sensilla (Sf). (B) Image from the proximal region of the flagellum indicating numbers of procumbent feathered setae (Pf), a standing feathered sensillum (Sf) and large beaked sensilla (B,l). (C) A scanning electron micrograph of a portion of the distal lateral flagellum from *P. clarkii*. Pairs of aesthetasc sensilla are indicated by asterisks. (D) A light micrograph of a portion of the lateral flagellum from a living specimen of *P. clarkii*. Note the optical transparency of the distal two-fifths of the aesthetascs, the region thought to be permeable to odorants. Scale bars: 100 µm. [A,B: DeF.M., unpublished results; C,D: reproduced from Mellon and Reidenbach (Mellon and Reidenbach, 2011).]

and mechanical information by neurons in the deutocerebrum has also begun, and the results are intriguing, if not presently very instructive.

Morphological and functional diversity of antennular sensilla

Despite the crucial importance of the antennular sensory armamentarium to crustacean lifestyles, it is only relatively recently that details concerning its structure and function have been seriously examined. Beginning in the 1970s, these studies were primarily concerned with the morphology and fine structure of aesthetasc sensilla found on the ventral aspects of the lateral antennular rami, or flagella, in spiny lobsters and crayfish (Tierney et al., 1986; Grünert and Ache, 1988; Mellon et al., 1989), and with the physiological properties of olfactory receptor neurons (ORNs) associated with the aesthetascs of clawed and spiny lobsters (Ache, 1972; Schmitt and Ache, 1979; Thompson and Ache, 1980; Johnson and Atema, 1983). The early electrophysiological studies from subdivided strands of antennular nerve axons largely ignored the possible contributions of chemoreceptor subtypes other than aesthetascs in response to chemical stimulation; thus, many of the early extracellular recordings from axons in the antennular nerve may have originated from non-aesthetasc chemoreceptors. It was only in the late 1980s that improvements in recording and dissection methodology permitted controlled focal recordings from identified olfactory receptor neurons in the spiny lobster (Schmiedel-Jakob et al., 1987) and this has since promoted highly detailed examination of the lobster ORN ion channels and the signal transduction pathways that regulate them (McClintock and Ache, 1989; Fadool et al., 1993; Hatt and Ache, 1994; Bobkov et al., 2010). Increasingly, however, current descriptive and experimental studies have also been focusing on diverse, non-aesthetasc setae on the antennules of lobsters as crucially important chemoreceptive structures in behaviors (Steullet et al., 2002; Horner et al., 2000; Schmidt and Derby, 2005). Most, if not all, of their associated chemoreceptor neurons occur within bimodal sensilla that have a mechanoreceptive function as well, similar to the bimodal mechano-chemoreceptor contact sensilla found on the walking legs and mouthparts of all decapods.

The diversity of non-aesthetasc receptors on the antennules of different crustaceans is exceedingly rich; even within single species, antennular sensilla diversity can range to half a dozen or more subtypes (e.g. Cate and Derby, 2001) and that of the crayfishes is no exception, with five types of setae being described for the Australian crayfish *Cherax destructor* (Sandeman and Luff, 1974). Fig. 1 illustrates most of the kinds of setae found on the lateral antennular flagellum of the crayfish *Procambarus clarkii* and which, with the exception of aesthetascs, are also found on the medial flagellum. They include non-innervated procumbent feathered setae, simple asymmetric setae, filamentous setae, small and large beaked sensilla, and standing feathered sensilla. With the exception of the aesthetascs, the standing feathered sensilla and, provisionally, the beaked sensilla, the functional properties of these structures are currently unknown.

Aesthetasc sensilla

Aesthetasc sensilla are found on the lateral flagellum of the crayfish antennules and are assumed to perform an olfactory function analogous to that of lobster aesthetascs. As shown in Fig. 1A,C,D, in *Procambarus* aesthetascs are arrayed usually in pairs on the annuli of the distal half of the lateral flagellum. Each aesthetasc sensillum is approximately 100 µm in length and about 15–20 µm in diameter at the base, and tapers gradually to a blunt tip. There is no pore at the tip. The distal 2/3 of the sensillum has a cuticle no more than 1 µm thick (Mellon et al., 1989) and is transparent to light (Fig. 1D); it is believed that this zone is permeable to dissolved odorants (Tierney et al., 1986). As in lobsters (Grünert and Ache, 1988; Schmidt and Ache, 1992), crayfish aesthetasc sensilla are each supplied by 150–300 bipolar ORNs having their cell bodies clustered within the associated sensory ganglion in that flagellar annulus. Their axons exclusively target the ipsilateral olfactory lobe (OL) glomeruli, possibly in a one-for-one pattern of connectivity (Mellon and Munger, 1990; Mellon and Alones, 1993). There is no anatomical or physiological evidence that the aesthetascs in any crustacean are also supplied by mechanoreceptor neurons. While the relatively much smaller size of the antennular flagella in *Procambarus* has so far precluded the type of focal electrophysiological recordings obtained from spiny lobster ORNs, intracellular recordings from local interneurons within the OLs of the crayfish deutocerebrum have indirectly implicated the ORNs as receptors for complex odorants and amino acids (Mellon and Alones, 1995). Furthermore, as will be discussed in a later section,
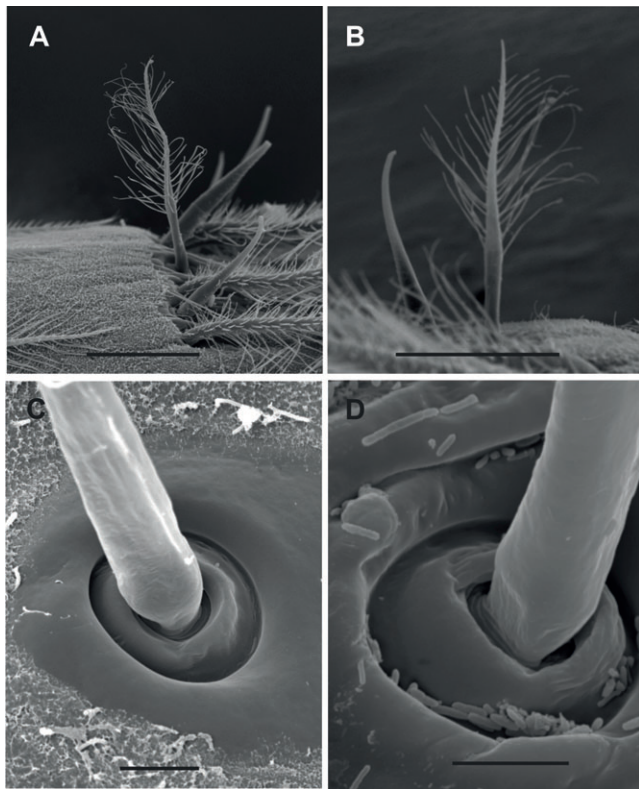
Fig. 2. (A,B), Scanning electron micrographs of standing feathered sensilla from the lateral antennular flagellum of *P. clarkii*. Note the relative lengths of the filaments along the sensillar shaft and the 'dog leg' feature. (C,D) Scanning electron micrographs of feathered sensillar bases. The hinge is a linear structure of thin, pliant cuticle that is continuous with the floor of the inner cup. Scale bars: A,B, 100 μm; C,D, 5 μm. [A: reproduced from Mellon and Christison-Lagay (Mellon and Christison-Lagay, 2008); B–D: DeF.M., unpublished results.]

some of these interneurons extend dendritic branches to the lateral antennular neuropil, a deutocerebral target for the axons of bimodal antennular sensilla in both decapods (Schmidt et al., 1992; Schmidt and Mellon, 2010) and stomatopods (Derby et al., 2003).

Standing feathered sensilla

Antennular standing feathered sensilla were first described (Mellon and Christisen-Lagay, 2008) as highly sensitive hydrodynamic near-field receptors having displacement firing thresholds as low as 0.02 μm to 10 Hz sinusoidal oscillations. These receptors, whose structural features are similar to those of near-field receptors of the crayfish branchiostegites (Mellon, 1963) and tailfan (Wiese, 1976; Douglass and Wilkens, 1998), are illustrated in Fig. 2. They constitute a first line of defense in the detection of hydrodynamic shear, including that attending predator approach, and it has been suggested that the summed activity from each of their associated pair of bidirectional sensory neurons can evoke escape tailflips triggered by the medial giant fibers in the brain (Mellon and Christisen-Lagay, 2008). Standing feathered sensilla are found on both the lateral and medial flagella of the crayfish antennules. They are, however, relatively sparsely arrayed linearly along both of the flagellar shafts, from the base to near the tip, with only 12 or fewer sensilla found on each flagellum. On the lateral flagellum, the feathered sensilla are located ventro-laterally; on the medial

flagella, they are arrayed ventro-medially. Morphologically, standing feathered sensilla are up to 150 μm long; they are characterized by a planar array of flat filaments on the distal 75% of the sensillar shaft, with the longest filaments located proximally. In terms of microscale fluid mechanics, this design feature makes sense: assuming that the viscous drag forces on the longer filaments will be greater than those on the shorter filaments, this arrangement logically takes advantage of the fact that the force required to deflect the sensillum through a critical angle at the hinge will, because of the lever arm, be smaller distally than at the base. Furthermore, because of the small Reynolds number of the sensillum (<1), the relatively thick fluid boundary layer dictates that fluid motion near the sensillar base will be less than that farther out along the sensillar shaft. As with the trichobothria of spiders, the longer sensors invade more of the boundary layer and are therefore exposed to higher fluid velocities (Humphrey and Barth, 2007). (Nonetheless, the response characteristics of any sensillum will also depend upon morphological features other than length, including both the torsional restoring constant and the damping constant of its cuticular connections.) The shaft itself has a distinctive 'dog leg' roughly 2/3 of the way from the base toward the tip (Fig. 2B). Standing feathered sensilla are extremely compliant to movements of the fluid medium, especially those in line with the flagellar axis (Mellon and Christisen-Lagay, 2008), a finding supported by scanning electron micrographic images of their basal hinge structure (Fig. 2C,D). The basal hinge is a thin, linear soft-cuticle attachment between the sensillum base and the inner socket on the flagellum. It is nominally aligned at 90 deg to the flagellar axis and the preferred plane of movement of the sensillum and its neuronal sensitivities.

Beaked sensilla

After the procumbent feathered setae, discussed below, the most numerous types of setae on either antennular flagellum in *Procambarus* are the long and short beaked setae, provisional sensilla, shown in Fig. 3A,B, and so named for their unusual tip structure. Beaked sensilla are roughly 15 μm in basal diameter and, similar to the standing feathered sensilla, can be 150 μm in length; the shorter versions vary between 50 and 75 μm in length. Beaked sensilla are less compliant than standing feathered sensilla, and therefore they probably are not normally receptors for near-field hydrodynamic stimuli. As discussed in greater detail below, there are anatomical reasons to conclude that beaked sensilla are bimodal contact chemo-mechanoreceptors, possibly similar in their responses capabilities to, for example, the hooded sensilla found on the antennular flagella of spiny lobsters (Cate and Derby, 2002a; Cate and Derby, 2002b). The arrangement of beaked sensilla along the shaft of the antennular flagella is not random (Fig. 3C). The long beaked sensilla are grouped together in pairs or threes at the dorsal and ventral anterior margin of each annulus, whereas the short beaked sensilla occur singly, and at a more lateral or medial location on the annulus. The functional reasons for these particular distribution patterns are not currently understood.

Distally, each beaked sensillum ends in a curved tip, resembling a goose beak, that has a cuticular structure visibly different from that of the sensillar shaft (Fig. 3B). Because there is presumptive evidence that these sensilla are bimodal chemo-mechanoreceptors, a question arises concerning the properties and function of this modified tip cuticle, and whether it may be permeable to chemical stimuli. Its crinkled and folded appearance suggests a modification to increase its surface area, possibly to enhance the access of dissolved chemical agents to internal chemoreceptor dendrites,
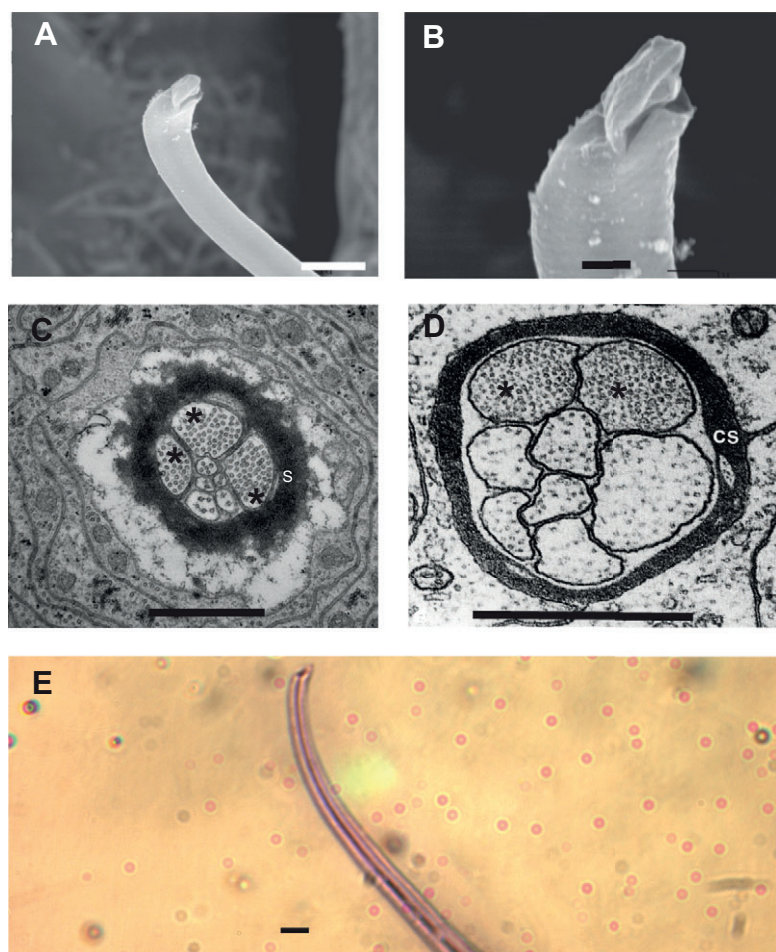
Fig. 3. (A,B) Scanning electron micrographs of beaked sensilla from the lateral antennular flagellum in *P. clarkii*. The magnification in B has been increased to illustrate the structure of the sensillar tip. (C) Transmission electron micrograph of a thin section through the base of a beaked sensillum, indicating eight distal dendritic profiles. Those marked with an asterisk have a high density of microtubule cross-sections and are presumed to belong to neurons having a mechanoreceptive function. S, scolopale. (D) Electron micrograph of a transverse section through the base of a hedgehog sensillum in the crayfish *Austropotamobius torrentium*. Asterisks mark distal dendrites having a high microtubule density [reproduced from Altner et al. (Altner et al., 1983) with kind permission from Springer Science and Business Media]. CS, cuticular sheath. (E) Light micrograph of a beaked sensillum from *P. clarkii* that was exposed to a 3% solution of Crystal Violet. The dye has penetrated the tip and filled the central canal of the sensillum. Scale bars: A, 3.5 μm; B–D, 1 μm; E, 10 μm. (A–C,E: DeF.M., unpublished results.)

whereas its permeability to the dye Crystal Violet (Fig. 3E) indicates small molecules may have similar access.

While a detailed ultrastructural analysis has not been performed with beaked sensilla, preliminary transmission electron microscopic examination of thin sections through their base consistently shows dendritic profiles similar to those in Fig. 3C; namely, three large diameter profiles with a relatively high density of microtubule cross-sections and five smaller profiles having a low density of microtubules. When these profile classes are compared with those of other crustacean sensilla having a known mechanoreceptor (Schmidt and Gnatzy, 1984) or bimodal chemo-mechanoreceptor properties, such as the hedgehog sensilla of crayfish walking legs (Altner et al., 1983) (Fig. 3D) and the hooded sensilla of the spiny lobster antennules (Cate and Derby, 2002a), the details can be used to infer function. The presence within the beaked sensilla of large-diameter dendritic profiles with a high microtubule density and an electron-dense structure known as a scolopale surrounding the distal dendrites are diagnostic features of arthropod cuticular sensory neurons having mechanoreceptive function. In contrast, the small diameter dendritic profiles with a low microtubule density continue distally to the sensillum tip, where in both the hedgehog sensilla and hooded sensilla cuticular modifications suggest a permeability to small molecules (Altner et al., 1983; Cate and Derby, 2002a). In consideration of these ultrastructural attributes (but with a noteworthy current lack of any physiological evidence), the beaked sensilla are assumed to be bimodal contact chemo-mechanoreceptors for sensing the substrate.

Miscellaneous setae

There are at least three additional morphological types of setae on the antennular flagella: procumbent feathered setae, filamentous setae and asymmetric setae. While the procumbent feathered setae (Fig. 1B) in other crayfish species are known not to be innervated (Bender et al., 1984), the functional status of neither the filamentous nor asymmetric setae is known. Filamentous setae, examples of which are shown in the scanning electron micrographs of Fig. 4A,C, are a very sparse population of long setae having a diameter at the base of about 10 μm and a length of over 200 μm. Furthermore, they are ornamented just near their tip by an array of long, thin (<0.5 μm) filaments or draglines, a feature that suggests these setae may act as hydrodynamic sensors. Asymmetric setae (Fig. 4B,D) are simple, tapering structures somewhat shorter than the aesthetascs, with which they are associated. They are about 10 μm in diameter at the base and less than 1 μm at the tip, which has a terminal pore. Their disposition and, to an extent, their morphology resemble the asymmetric sensilla of the spiny lobster antennules after which they have been named. In lobsters, asymmetric setae are believed to be bimodal chemo-mechanoreceptor sensilla, as behavioral studies indicate that they are solely responsible for triggering reflex antennular grooming in the presence of threshold concentrations of L-glutamate (Schmidt and Derby, 2005). Furthermore, a scolopale is present around the outer dendrites of associated sensory neurons at the base of the lobster asymmetric setae, implicating them as mechanoreceptors (Schmidt and Derby, 2005). It is currently unknown whether the asymmetric setae of the crayfish antennule mediate grooming or any other form
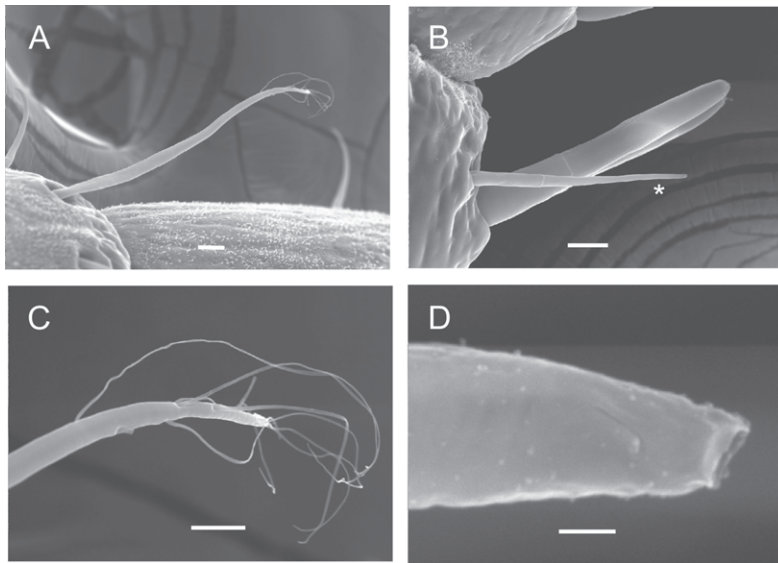
Fig. 4. (A,B) Scanning electron micrographs of, respectively, a filamentous seta and an asymmetric seta (asterisk) from the lateral flagellum of *P. clarkii*. (C,D) The tip regions of both setae at higher magnification. Scale bars: A, 20 μm; B,C, 10 μm; D, 0.5 μm. (DeF.M., unpublished results.)

of behavior, nor is there any extant evidence for their sensory innervation.

Integration of antennular input signals by the brain

What is the destination of information from all of the diverse antennular sensilla? Where does it end up in the brain, and how is it used to maximize the picture available to the crayfish of its chemical, tactile and hydrodynamic environments? All flagellar sensory axons in crustaceans terminate within the deutocerebrum, delineated with its subdivisions by the dotted line in Fig. 5A, a highly simplified dorsal view diagram of the brain of *P. clarkii*. [The structures labeled accessory lobes (AL) receive secondary information from the OL, the visual centers in the protocerebrum, and the tactile/chemoreceptor inputs from the tritocerebrum. They do not receive primary input from any sensory source (Sandeman et al., 1995), and while they are of undoubted importance in the integration of antennular inputs with other sensory modalities, they will not be considered further in this Commentary.] Although fluorescent dye tracing of axonal afferents from the crayfish antennules has not been performed, tritiated leucine uptake experiments have revealed axons of olfactory receptor neurons terminating within the OL glomeruli (Mellon et al., 1989; Mellon and Munger, 1990) and, by analogy with the spiny lobster (Schmidt et al., 1992) and stomatopods (Derby et al., 2003), the lateral antennular neuropils (LAN) and the median antennular neuropil (MAN) apparently receive inputs from all but the aesthetasc sensilla on both flagella. Consideration of central interactions of these inputs is addressed in the final section of this Commentary.

Active chemoreception: hydrodynamic–olfactory co-operation in the detection of odorant stimuli

Near the bottom of lakes and streams, where crayfish feed, fluid flow can be generally turbulent, especially in the presence of certain substrate types (Moore and Grills, 1999). Within this benthic region, therefore, dissolved odorants are imbedded in and distributed by intermittent, chaotic eddies having unpredictable temporal and spatial structure (Weissburg, 2000; Webster and Weissburg, 2001; Atema, 1996; Grasso and Basil, 2002). The moment-to-moment detection of hydrodynamic discontinuities may therefore be of crucial importance in odorant capture. At the same time, antennular flicking enhances the detection of dissolved odorants by shedding,

or at least by decreasing, the fluid boundary layer around the individual aesthetasc sensilla, thereby enhancing their exposure to novel water samples and their imbedded chemical agents through molecular diffusion (Reidenbach et al., 2008; Mellon and Reidenbach, 2011). Turbulent eddy fronts within the water column are therefore cues to the possibility of novel odorant exposure, and they trigger antennular flicking. They are detected by hydrodynamic sensors on the crayfish lateral antennular flagellum (Mellon, 1997), recently specifically identified as the standing feathered sensilla (Mellon and Christisen-Lagay, 2008), and which are sufficient to directly evoke reflexive flicking of the lateral flagellum (Mellon and Abdul Hamid, 2012). In fortuitous examples, a brief (2 ms) mechanical stimulus to even a single standing feathered sensillum is adequate to trigger the flick reflex at a consistent, if rather long, response latency. When the same stimulus is applied to the flagellum as a whole, thereby presumably simultaneously exciting the entire array of feathered sensilla, the response latency decreases to minimal values, suggesting that temporal summation of the inputs from the array of 10–12 feathered sensilla at their central targets minimizes flick response time, arguably a crucial factor in odorant capture. Although it is understood that odorant inputs by themselves can also trigger antennular flicking (Daniel and Derby, 1991), the response latency of the tiny olfactory afferents is 30–100% longer than that for the hydrodynamic receptors (see below), suggesting that, even in the presence of strong odorant concentrations, the initial trigger for flicking is mechanical. Therefore, the contribution of near-field sensors in evoking a specific behavior appears to be an important cooperative factor in odorant detection. This conclusion is supported by observations that active flicking is terminated when the velocity of fluid flow past the antennules exceeds a critical value (Moore and Grills, 1999) (G. McGlaughlin and DeF.M., unpublished observations).

Active chemoreception: is there co-operation between the asthetascs and distributed antennular chemoreceptor sensilla?

My anecdotal observations raise the possibility that contact chemoreceptors on the antennules are used to investigate an odorant source on the substrate that was initially detected at a distance by the aesthetasc sensilla. In aquarium settings, I have observed *P. clarkii* individuals searching for tetramin pellets that had been dropped into the water, to sink to the bottom some distance away
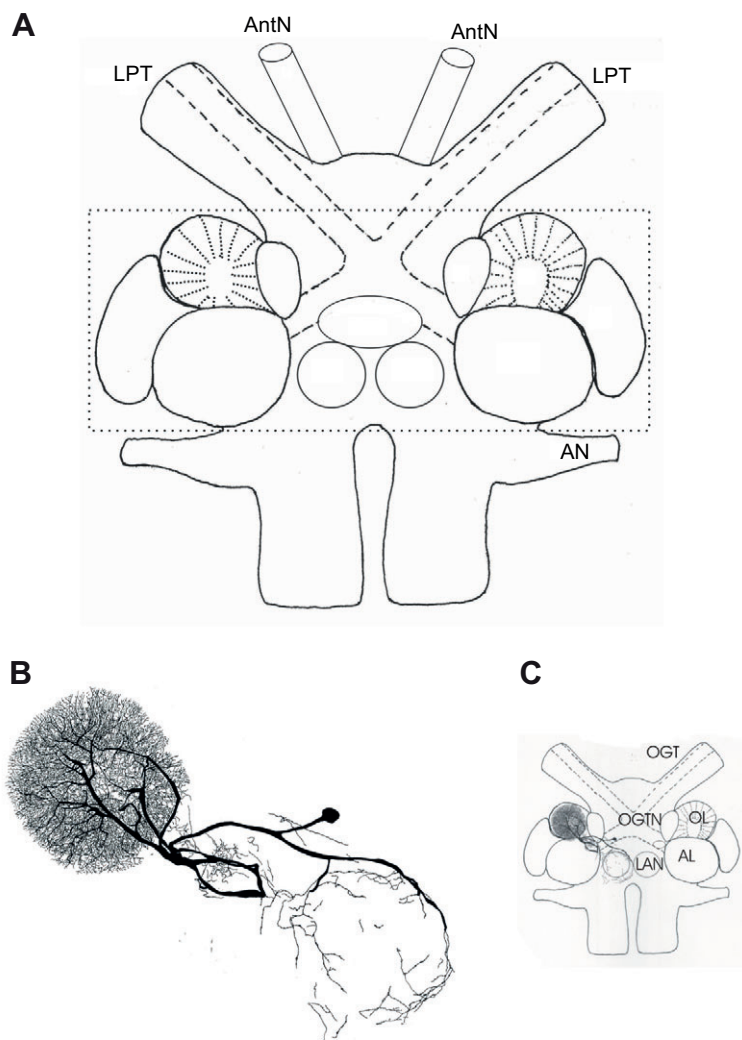
A

B

C

Fig. 5. (A) Highly simplified diagram of a dorsal view of the brain of *P. clarkii*. The area enclosed by the dotted rectangle roughly indicates the deutocerebrum and some associated structures. AL, accessory lobe; AN, antennal nerve; AntN, antennular nerve; LAN, lateral antennular neuropil; MAN, median antennular neuropil; LPT, lateral protocerebral tract; OL, olfactory lobe; 10 and 11, neuronal cell body clusters of the deutocerebrum. (B) Camera lucida rendering of a Type I local deutocerebral interneuron from the brain of *P. clarkii*. (C) The same Type I neuron superimposed to scale on the brain; a dense dendritic arborization occurs within the left OL and, to a lesser extent, within the left LAN. One output pathway of Type I neurons is believed to be in the olfactory globular tract neuropil (OGTN). Dashed lines indicate the olfactory globular tract (OGT) within which run axons of projection neurons from the OL and AL to centers within the lateral protocerebrum (not shown). [B: reproduced from Mellon and Alones (Mellon and Alones, 1995).]

from the animal. After initial detection, involving an increase in antennular flicking frequency, successful searches by the animal resulted in the pellet being grasped and manipulated by the chelate pereiopods and maxillipeds while at the same time the antennular flagella were depressed to make physical contact with the now-disintegrating pellet. While obviously speculative at this point, the behavior is suggestive that contact chemoreceptive sensilla on the flagella were being enlisted to report the identity of the odorant signals that initiated the search, perhaps thereby actively suppressing search behavior. Feeding is largely reflexive and autonomous once chemoreceptors on the pereiopods and mouthparts come into contact with food; indeed, animals happily pass food from the pereiopods to the mouthparts even in the absence of a brain (Huxley, 1896). And while it is currently unknown whether there are inputs from thoracic contact chemoreceptors to the brain, there can be little question that both olfactory receptor neurons from the aesthetascs and non-aesthetasc chemoreceptor neurons project to centers within the deutocerebrum, where the potential for convergence of inputs from both sources onto common local interneurons must be considered.

Neural integration of olfactory and hydrodynamic inputs from the crayfish antennule

Axons of all sense organs from the crayfish antennular flagella, regardless of modality, terminate within the OL, the LAN or the MAN. At least two types of local deutocerebral sensory interneurons extend dendritic branches to both the OL and the LAN and, thereby, can potentially integrate the inputs received over both sets of afferent pathways (Fig. 5B,C). As discussed below, there is now solid experimental evidence for deutocerebral neural integration of inputs from the aesthetasc sensilla and from as yet unidentified hydrodynamic sensilla on the lateral antennular flagellum (Mellon, 2005; Mellon and Humphrey, 2007).

A major class of local sensory interneuron within the deutocerebrum, Type I, illustrated in Fig. 5B, has extensive multiglomerular arborizations in the OL and also dendritic ramifications in the ipsilateral LAN (Mellon and Alones, 1995). Sharp intracellular electrode recordings can be obtained from large diameter dendritic branches of Type I neurons within the OL. A variety of odorants (amino acids, glucose, tetramin) applied to the lateral (but not the medial) antennular flagellum evoke excitatory postsynaptic potentials (EPSPs) and spike trains in Type I interneurons in a dose-dependent manner (Mellon and Alones, 1995; Mellon, 2005). A second class of local interneuron (Type II, not illustrated, and more difficult to record from) with multiglomerular dendritic arborizations in the OL and having additional inputs in the LAN, is also excited by hydrodynamic input but is inhibited in a dose-dependent manner by the same classes of odorants that excite the lateral antennular flagellum (Mellon and Alones, 1995). As shown by the electrical recordings in Fig. 6A,C,
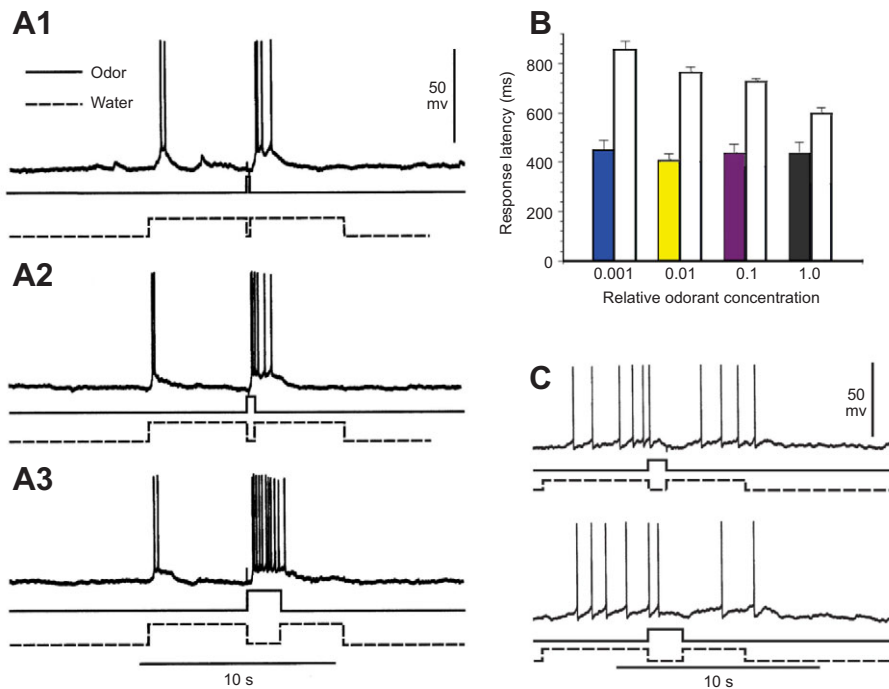
A1

A2

A3

B

C

Fig. 6. (A) Intracellular electrical recordings of the activity of a Type I deutocerebral local interneuron in response to the onset of fresh water (dashed lines) or tetramin odorant (solid lines) pulses past the ipsilateral lateral antennular flagellum. The duration of the odorant pulses increased from A1 to A3. The records were obtained after 2 min periods of disadaptation. (B) Bar graphs of response latencies of a Type I neuron to fresh water (filled bars) or seamlessly introduced odorant (open bars) at four different odorant concentrations. Even with undiluted odorant (0.05% tetramin) the response latency to water alone was only 2/3 that of the latency to odorant. [All records reproduced from Mellon (Mellon, 2005).]

both Type I and Type II local interneurons are excited by fluid flow along the lateral antennular flagellum, transiently in the case of Type I cells and continuously for Type II cells. Moreover, the efficacy of the hydrodynamic response in Type I cells is critically dependent upon the direction of fluid movement along the flagellum. Fluid flow from proximal to distal is more effective than that in the opposite direction (Mellon and Humphrey, 2007). This experimental observation is consistent with the theoretically modeled fluid flow along the flagellum during flicking behavior (Humphrey and Mellon, 2007). Therefore, during antennular flicking, there exists the possibility that the central response of Type I neurons to imbedded odorants is potentiated by fluid movements along the lateral flagellum. Cooperation between olfaction and hydrodynamic sensors in foraging has been observed in other phyla; for example, dogfish deprived of their lateral line inputs have considerable difficulty in localizing an odorant source under conditions of turbulent fluid flow (Gardiner and Atema, 2007). The response of Type I cells to hydrodynamic stimulation habituates rapidly, however, and can take up to 60 s to recover its initial sensitivity (the cause of the habituation, whether central or peripheral, is unclear at this point). But, in experiments with non-habituated preparations designed to include odorant with the onset hydrodynamic stimulation often generated electrical responses that were greater than those occurring when odorants were introduced seamlessly into an ongoing stream of fresh water (Fig. 7). While this did not happen in every Type I cell we recorded from, on those occasions where it was present it increased the rate of rise of the odorant-evoked EPSP and generated up to twice the spike number as well as increasing spike frequency (Mellon, 2005). Therefore, this phenomenon could play a role in the detection of low concentrations of odorant when the aesthetasc array is suddenly exposed to an eddy front. During repetitive flicking, the habituation of the response to hydrodynamic input neurons would suggest that this synergy is no longer available. The identity of the hydrodynamic receptors exciting Type I neurons is currently unknown. The standing feathered sensilla characterized above are not good candidate sensors for this response, because their

bidirectional sensitivity is at odds with the finding discussed above that the response of Type I cells to fluid flow is polarized.

What, then, is the functional significance of the Type II neurons that are continuously excited by fluid flow but actively inhibited by odorants in a dose-dependent manner? Type II cells clearly are a parallel system to those designated as Type I, but they would be most effective in different circumstances; namely, during periods when the hydrodynamic responses in Type I cells have suffered adaptation. Type II cells would be expected to show the greatest sensitivity to low concentrations of odorants when they are
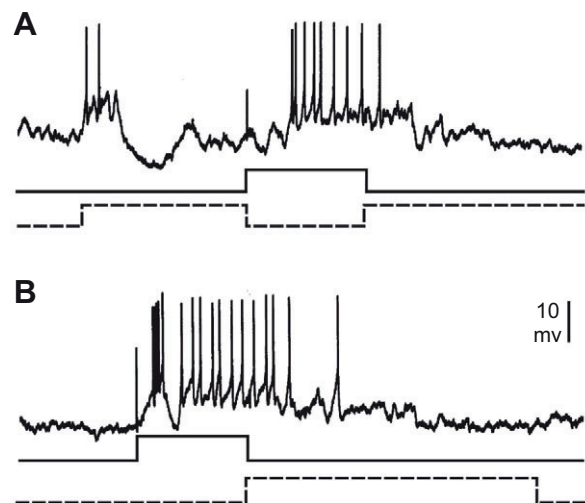
A

B

Fig. 7. Responses of a Type I local interneuron in *P. clarkii* to different presentation protocols of water and odorant. (A) Fresh water was initiated past the lateral antennular flagellum for 3 s, at which point odorant was seamlessly added to the flow stream for 2 s. (B) A 2 s pulse of odorant was initiated in a flow past the flagellum first, followed by a 5 s pulse of fresh water. When fluid flow and odorant were initiated together, the response of the neuron was increased in terms of spike number and frequency. [Reproduced from Mellon (Mellon, 2005).]

responding with the highest spike frequencies; that is, in the presence of strong hydrodynamic inputs during ambient water current movement, times during which Type I cells will be driven only by aesthetasc input. It can be reasonably argued, therefore – though perhaps, given the paucity of available experimental information, not as forcefully as one might wish – that there exist both phasic and tonic deutocerebral systems for detecting imbedded odorants and that these are co-dependent. The phasic, Type I system would operate at peak efficacy to detect odorants imbedded in eddies in relatively calm and stable situations when bulk fluid flow is slow or non-existent, whereas the tonic system would operate during periods when there exists consistent, strong water flow past the antennules. Presumably, the task of both systems is to alert the crayfish to the presence of interesting odorant traces and to initiate appropriate searching behaviors, but the precise cues used by foraging crustaceans is not well understood. Both the time-averaged number of concentration peaks within a turbulent plume downstream of an odorant source and the slope of the changes in concentration within an approaching peak have been suggested as possible cues in locating the source (Atema, 1996). An especially enlightening study by Webster and Weissburg (Webster and Weissburg, 2001), however, has determined that neither cue is likely to be usable by a foraging crustacean, as time averaging is not practical at durations of less than about 200 s – far too long to account for the much shorter times required by some foraging crustaceans to detect and reach an odorant source (Weissburg, 2000) – and resolution of concentration changes within an approaching peak slope require sampling times of no longer than 10 ms, roughly 20 times shorter than the observed optimal integration time for crustacean aesthetasc receptors (Gomez and Atema, 1996). The situation is further complicated by the fact that the gross structure of an odorant plume is determined by both the height of the source above the substrate and the velocity of odorant release in comparison with the velocity of the surrounding bulk fluid flow (Webster and Weissburg, 2001), and a downstream foraging crustacean would be unlikely to determine any of these details. In contrast, there is circumstantial evidence that some crustaceans, such as the swimming crab *Callinectes*, use a strategy similar to insect amenotaxis; in such odor-gated rheotaxis, the forager continuously moves upstream against the bulk flow, casting back and forth across the stream, and turning back into it when it either loses contact with or senses a sudden reduction in the strength of the odorant plume (Weissburg, 2000).

Is there neural integration of inputs from bimodal contact sensors?

Are the chemosensory and mechanosensory inputs from individual bimodal sensilla on the antennules integrated synergistically within the central brain centers? Both modalities are contact sensors, and a logical argument can be developed that their respective inputs act together at their central targets, perhaps in an obligate manner. For example, the chemoreceptive input might have little effect centrally unless there was also simultaneous input from the mechanoreceptive pathway with which it is paired in the sensillum. Spurious responses to water-borne chemical agents would thereby be avoided. This question seems not to have been examined previously, even though it may provide a reasonable explanation for the presence of bimodal cuticular sensilla in many arthropods. If in fact a central synergy mechanism as suggested above does exist in crayfish, it would further functionally separate the central actions of distributed antennular chemoreception from those of aesthetasc (olfactory) chemoreception, assuring that inputs from the former produce little central impact unless accompanied by associated mechanical stimulation.

Questions, moving forward

Additional questions emerge from the studies and observations reviewed above. For example, what other types of local deutocerebral interneurons are involved in the integration of chemoreceptor and mechanoreceptor inputs in the crayfish? At the periphery, the presence of different kinds of bimodal sensilla on the antennules and appendages begs the question of whether they are all sensitive to the same chemical stimuli, and whether any of their sensitivities overlap with those of the aesthetasc olfactory sensilla. Certainly, in the case of the asymmetric sensilla on the spiny lobster (Schmidt and Derby, 2005), which are sensitive exclusively to the amino acid L-glutamate, the existence of highly specialized narrowly tuned chemoreceptors must be recognized. Questions can also be asked about the functional significance of the different morphological classes of bimodal sensilla on the various appendages. Why are they so different? Possibilities include range fractionation in responses to different fluid flow velocities, as well as the necessity for robustness in the sensilla on the pereiopod dactyls, as they are exposed to the abrasive consequence of residing on the tips of walking appendages, whereas those on the antennules are exposed to substrate contact only briefly, and with far less pressure, when the crayfish depresses them. Another intriguing question concerns the significance of the different spacing patterns and numbers among aesthetascs (and their associated sensory neuron complements) in different species of crustaceans. What advantages do crayfishes accrue for having a sparse, very distributed pattern of aesthetasc placement, in contrast to those on antennules of lobsters crabs and hermit crabs, which exist at high density within compact tufts? These questions and others to do with the central actions of bimodal inputs are ripe for physiological inquiry. The neuropil centers and cell clusters of the deutocerebrum comprise more than 50% by volume of the crayfish brain; moreover, it is the only region of the crayfish brain where adult neurogenesis has been shown to occur (Sullivan et al., 2007), testaments to the crucial importance of antennular input. Crustaceans are an ancient, highly successful arthropod group. Understanding their chemo-mechanoreceptive integrative mechanisms can provide an important window into this success. It is clear, however, that this exploration has only just begun; the future, therefore, is rich with possibilities for new information and a more comprehensive understanding of the selective value of antennular sense organs in crayfish behavior.

Glossary

Aesthetascs
The olfactory sensilla found on the lateral antennular flagellum in crustaceans.

Antenna, or second antenna
A long, uniramous, movable sense organ projecting rostro-laterally from the crustacean cephalothorax.

Antennule, or first antenna
A bifurcate, movable sense organ projecting rostro-medially from the head region in crustaceans.

Boundary layer
The surface layer of fluid surrounding an object subject to laminar flow conditions, within which the rate of fluid flow varies monotonically, from zero at the object's surface to that of the bulk of the moving fluid.

Chela
The claw on crustacean pereiopods.

Dactyl

The distal segment of the periopods, or the movable segment of the chela.

Deutocerebrum

A central region of the crustacean brain, sandwiched between the rostral protocerebrum and the caudal tritocerebrum.

Glomeruli

Columnar structured neuropil of the olfactory lobes in the crayfish deutocerebrum, within which axon terminals from aesthetasc sensilla make synaptic connections with local interneurons and olfactory projection neurons.

Microtubule

Tubular, distal extensions of the ciliary structure within a sensory neuron dendrite.

Neuropil

Dense areas of dendritic and/or axon terminals making synaptic contacts within the central nervous system.

Pereiopods

The thoracic, walking legs of malacostracan crustaceans, including the large claws.

Reynolds number

The ratio of inertial to viscous fluid forces, and derived from the equation $Re=UL/\nu$ where U is velocity, L is the diameter of the object being considered and ν is the kinematic viscosity of the fluid.

Rheotaxis

Response of an organism to ambient fluid flow.

Scolopale

An electron-dense structure surrounding a region of the outer dendrite within some arthropod cuticular mechanosensilla and believed to assist in anchoring the dendrite to the wall of the sensillum.

Sensilla

Setae having a known sensory function.

Setae

Hair-like cuticular projections of the arthropod cuticle, usually supplied by chemosensory and/or mechanosensory neurons.

Statocysts

Paired sense organs on the basal segments of the antennules that determine relative gravitational direction and angular acceleration.

Tropotaxis

Movement of an organism in response to variations in two classes of stimuli; for example, fluid flow and odorant concentration.

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