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Directional asymmetry in responses of local interneurons in the crayfish deutocerebrum to hydrodynamic stimulation of the lateral antennular flagellum

DeForest Mellon, Jr[1,*] and Joseph A. C. Humphrey[1,2]

[1]*Department of Biology and* [2]*Department of Mechanical and Aerospace Engineering, University of Virginia, Charlottesville, VA 22903, USA*

*Author for correspondence (e-mail: dmbd@virginia.edu)

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Summary

We have recorded spiking responses from single, bimodally sensitive local interneurons (Type I) in the crayfish deutocerebrum to hydrodynamic and odorant stimuli flowing in two directions past the lateral antennular flagellum. Changing the direction of seamless introductions (meaning, with minimal variations of fluid velocity magnitude) of odorant flow past the flagellum, from proximal→distal to distal→proximal, did not consistently affect the dose-dependent responses of Type I neurons. By contrast, changing the direction of an abruptly initiated flow of water (or odorant) past the flagellum resulted in consistently larger numbers of spikes in response to this hydrodynamic stimulation when the flow direction was proximal→distal. This response asymmetry is discussed in relation to its possible relevance regarding antennular flicking behavior. The putative involvement of flagellar hydrodynamic receptors, the beaked hairs, and the hydrodynamic flow asymmetries they are exposed to, are examined theoretically in the accompanying paper.

Key words: crustacean, antennule, hydrodynamic.

Introduction

In aquatic as well as terrestrial environments, odors are dispersed primarily by advection and eddy turbulence, with passive diffusion playing only a minor role (Dusenberry, 1992). Information about local fluid movements is thus of critical importance to organisms that depend upon olfaction to locate food, mates or nearby predators (Dethier, 1980; Reeder and Ache, 1980; Weissburg and Zimmer-Faust, 1994; Weissburg, 2000; Atema, 1996; Moore and Grills, 1999; Breithaupt and Eger, 2002; Johnson and Atema, 2005; Stensmyr et al., 2005; Wyeth and Willows, 2006; Wyeth et al., 2006), and it is not surprising that most animals are well equipped with sense organs capable of detecting fluid motion. The central nervous mechanisms by which inputs from these structures and from olfactory receptors are integrated, however, remain largely unknown. A recent study (Mellon, 2005) suggests that one place where integration of hydrodynamic with olfactory input occurs is in large local interneurons in the olfactory lobes (OL) of the crustacean deutocerebrum. Especially in those cells previously designated as Type I interneurons (Mellon and Alones, 1995; Mellon, 1996), which are excited in a dose-dependent manner by odorants sensed through the antennules, hydrodynamic stimulation of the external antennular flagellum is excitatory and, in at least some of the individual neurons examined, amplified the spiking responses due to odorant exposure (Mellon, 2005).

The major olfactory organs in crustaceans are the lateral antennular flagella, which bear chemoreceptors referred to as aesthetascs (Fig. 1A,B). In crayfish these are blunt setae arrayed along the ventral surface of the lateral flagellum. Each sensillum contains the distal dendrites of about 170 olfactory receptor neurons (ORN) whose axons course through the antennular nerve to the ipsilateral OL (Mellon et al., 1989). As shown in Fig. 1B, in the crayfish *Procambarus clarkii* 2–4 aesthetasc sensilla are arrayed on each annulus along the ventral surface of the distal one-half of each lateral flagellum (Mellon et al., 1989). Additional types of setae – beaked hairs, standing feathered hairs and filamentous hairs – are found along the flagellum, and some of these may have a dual chemoreceptive–mechanoreceptive function, as has been described for a number of different types of non-aesthetasc antennular sensilla in the spiny lobster (Cate and Derby, 2001; Cate and Derby, 2002; Schmidt and Derby, 2005). Although no published worked exists concerning the sensory physiology or internal fine structure of non-aesthetasc chemoreceptive sensillum types in the crayfish, unpublished transmission electron micrographic studies indicate that the beaked hairs are supplied at their bases by dendrites of two diameter classes (DeF.M., unpublished observations), possibly associated with both chemosensory and mechanosensory neurons, respectively.

The direction of water movement during flicking with respect to individual sensilla on the lateral flagellum in *Procambarus* is influenced by its upwardly curved morphology, from which non-aesthetasc, beaked setae project at an angle of approximately 45° with respect to the local tangent to the flagellar surface [see discussion relating to fig. 2 in the

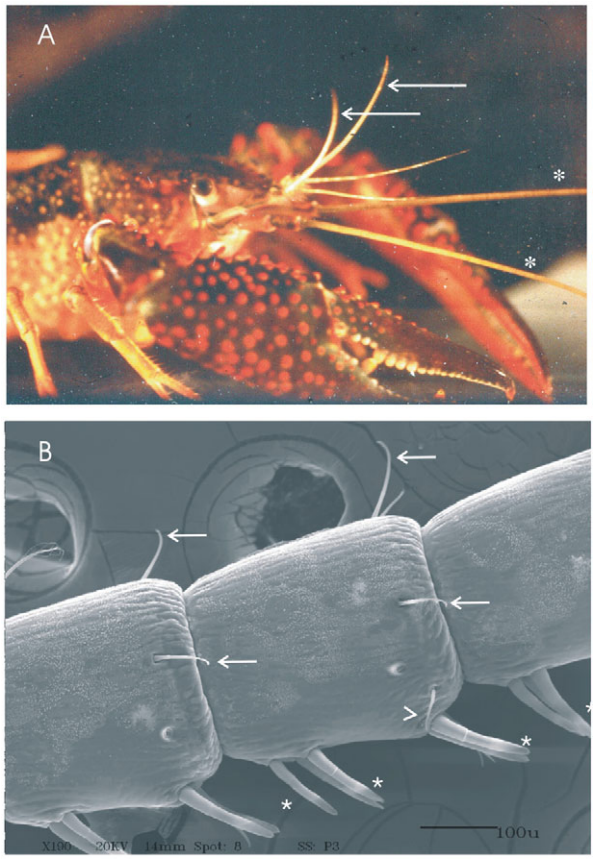

Fig. 1. (A) Anterior aspects of *Procambarus clarkii*, showing the long paired second antennae (asterisks) and the biramous antennules, the lateral flagella of which are indicated by white arrows. There is an upward curve to each lateral flagellum, which in this large animal is 2.5 cm long. (B) Several annuli of a lateral antennular flagellum of *P. clarkii*. Aesthetasc sensilla (asterisks) are arrayed ventrally on each annulus of the distal half of the flagellum, usually in groups of 2–4. At least three other types of sensillum occur on the medial and lateral flagella, including the most numerous class, the beaked hairs, indicated here with white arrows, and a standing feathered hair (white caret).

accompanying paper (Humphrey and Mellon, 2007)]. These structures are thus well-positioned to sample water movements, especially those directed toward the crayfish from its anterior aspect. Detailed analyses and discussions, with supporting figures, of various hydrodynamic considerations relating to the flow past the free-flicking crayfish antennular flagellum and the flow past a flagellum fixed in a tube are presented in the following companion paper (Humphrey and Mellon, 2007). The flexibility of the distal half of the lateral flagellum in *Procambarus* assures that its upward curvature will be accentuated to the point of being deflected caudally by even moderate advective flow approaching the front of the animal. This means that water/odorant will move past the deflected flagellum in a direction normal to it over the proximal two thirds of its length, and increasingly parallel to it along the distal third towards the tip, as discussed in relation to fig. 1 in the accompanying paper (Humphrey and Mellon, 2007).

Antennular flicking is a behavior exhibited by all aquatic and terrestrial decapod crustaceans and is believed to enhance the detection of odors (Snow, 1973; Snow, 1975; Price and Ache, 1977; Schmitt and Ache, 1979). In spiny lobsters, flicking temporally enhances the spike responses of ORNs to stable or slowly rising odorant concentrations (Schmitt and Ache, 1979), but the physical mechanisms through which this enhancement occurs are not completely understood. Koehl et al. (Koehl et al., 2002; Koehl, 2005) have reported from dynamic scaling and Particle Image Velocimetry experiments that the downstroke of antennular flicking observed in the lobster disrupts the boundary layer surrounding individual aesthetasc sensilla, whereas the upstroke entraps the odor-laden water sample resulting from this disruption. Further support for this conclusion comes from studies of antennular movements in stomatopods (Mead and Koehl, 2000). Freshwater crayfish also flick their antennules. However, the much smaller surface density of aesthetascs on the crayfish antennule (2–4 per annulus) compared to the lobster antennule (16–20 per annulus) raises the possibility that a different functional mechanism may be involved.

Flicking undoubtedly generates hydrodynamic forces that impinge upon the aesthetascs and other classes of sensilla on the lateral flagellum, and a question therefore arises concerning the possible directional sensitivity of these sensory structures to fluid flow along the antennular axis. Accordingly, we explored the sensitivity of Type I OL interneurons [cells with somata in cluster 11 of the crayfish brain (Sandeman et al., 1992) and having dendritic inputs in both OL and LAN (Mellon and Alones, 1995)] to fluid flow along the lateral antennular flagellum of *Procambarus clarkii* in the proximal-to-distal (P→D) and the distal-to-proximal (D→P) directions. An asymmetry is observed in the response of these neurons to the direction of antennular hydrodynamic stimulation, the result of a selective sensitivity of one or more types of antennular mechanoreceptors to the direction of fluid movement past the flagellum. The present paper documents this asymmetry. Furthermore, calculations presented in the accompanying paper (Humphrey and Mellon, 2007) indicate that drag and torques experienced by the flagellum within the reversing olfactometer, respectively, during fluid flow in the P→D and the D→P direction correlate well with drag forces and torques experienced over the bulk of the flagellum during downward and upward flick cycles. The experimental data and their theoretical interpretation suggest that, whatever enhancement of odorant detection may occur through peripheral factors affecting the aesthetasc sensilla during antennular flicking, central mechanisms ensure that hydrodynamic consequences of flicking will supplement ambient chemical signals.

Materials and methods

Adult *Procambarus clarkii* Girard, approximately 50 mm in carapace length, were obtained from suppliers in Louisiana (Atchafalaya Biological Supply, Raceland, LA, USA and Carolina Biological Supply Co, Wabun Labs, Wabun, LA, USA). Crayfish were communally housed in large fiberglass tubs of filtered, circulating freshwater at 20°C until used for experiments. They were fed on *Elodea* and crayfish chow twice a week and were kept under a light/dark regime of 12 h:12 h L:D.

Crayfish were prepared for recording by placing them in crushed ice for 15 min, then quickly decapitating them by

cutting around the cephalothorax just anterior of the cervical groove. The isolated head was prepared as described previously (Mellon, 2005). Basically, the head was pinned to the Sylgard™ floor of a suitable Lucite™ recording chamber, with the lateral antennular flagellum on one side inserted into a special, reversible-flow olfactometer (see below and Fig. 2). The base of the flagellum was sealed with Vaseline™ to isolate its shaft within the olfactometer from the saline in the recording chamber. The recording chamber was then flooded with chilled crayfish saline having the following composition (in $mmol^{-1}$): NaCl, 205; KCl, 5.4; $CaCl_2 2H_2O$, 13.6; $MgCl_2 7H_2O$, 2.7; $NaHCO_3$, 2.4. The pH of the saline was adjusted to 7.4 using HCl. Small glass cannulae were placed within the cor frontale, communicating with the brain's median artery, and in the lateral cephalic artery ipsilateral to the flagellum within the olfactometer. The cannulae were connected to a reservoir of chilled, oxygenated crayfish saline that was pressurized to ensure an adequate flow through the brain and the antennule. The temperature of the saline within the recording chamber was (17°C).

Reversible-flow olfactometer

Fig. 2 is a diagram of the plumbing and switches used to control fluid movements through the reversible-flow olfactometer. The olfactometer itself was constructed from a section of 1.25 cm diameter Lucite™ rod and essentially consisted of a cylindrical chamber 2.4 mm in diameter. Two ports, each 1.0 mm in diameter, were drilled through the ventral aspect of the cylinder 25 mm apart. When sealed at both ends, the volume of the empty olfactometer chamber was 113 μl; however, with the flagellum in place the free volume probably was 3.5–5% smaller.

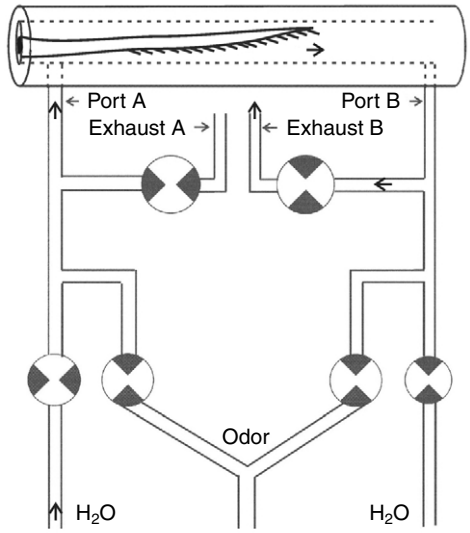

Fig. 2. Diagram of the plumbing circuits associated with the reversible-flow olfactometer. A standard crayfish head preparation was secured in a recording chamber (not shown), and the lateral antennular flagellum on one side was inserted into the olfactometer and sealed at the base with Vaseline™. Fluid could be introduced to the olfactometer either through port A, at the base of the antennule, or alternatively through port B, beyond the tip of the flagellum. Solenoid switches were controlled so that when either water or odorant entered through one port, the exhaust to the opposite port was simultaneously opened. The diagram shows an instance when water is entering port A, flowing through the olfactometer past the flagellum in the P→D direction, and out through exhaust B (arrows).

Fluid flow through the olfactometer, driven by gravity from reservoirs positioned approximately 75 cm above the preparation, was controlled by solenoid valves (Lee Co., Westbrook, CT, USA) activated by electronic stimulators (Astro-Med, Inc., West Warwick, RI, USA) and timed by a multi-channel pulse generator (World Precision Instruments, Sarasota, FL, USA) the outputs of which, along with the neuronal recordings, were monitored by a data acquisition program (Pclamp 8.2, Axon Instruments, Burlingame CA, USA). Typically, a 10 s pulse of dechlorinated tapwater was permitted to flow through either port A or port B, with the opposite port simultaneously being switched to its exhaust mode. Following a delay of 2–4 s, odorant solution was seamlessly exchanged for the water flow at the entry port, usually for a period of 4 s, after which water again flowed through the port for the remainder of the 10 s time window. In practice, during acquisition of data from brain neurons, fluid entry (water and odorant) into either port A or port B were alternated with each other. All tests were separated by a minimum interval of 2 min, during which time the olfactometer was flushed with fresh dechlorinated water for 30 s. Before each experimental session, reservoir heights above the preparation were adjusted to ensure similar flow rates for water and odorant; in practice, this was not always achieved precisely, possibly due to episodic variations in the operation of the solenoid valves. Larger variations among flow rates measured with different preparations may have been due to different sizes of the flagella being tested. At the end of each experiment, prior to removing the isolated head from the recording chamber and with the antennular flagellum *in situ*, the flow rates of water and odorant through the olfactometer in both directions were measured by collecting and measuring the volume of exhaust fluid during a 10 s pulse from each reservoir. These numbers were normalized across all experiments to provide a measure of the mean difference and variation in the D–P normalized flow rate.

Preparation of odorant solutions

Odorant solutions were prepared by making a stock solution of either Tetramin™ or Prime Reef™ as a 1% w/v solution in deionized water. After filtering the stock was divided into 5 ml portions and frozen at –20°C. For use, a 'standard' odorant solution was prepared by dissolving a 5 ml aliquot in 45 ml of dechlorinated freshwater to make a 0.1% solution of the odorant. This standard was further diluted 10×, 100× or 1000× to obtain an odorant intensity–response series on Type I neurons.

Electrical recording

Prior to recording, the dorsal surface of the brain was desheathed with fine forceps to reveal the OL ipsilateral to the antennule being tested. Sharp glass capillary microelectrodes were pulled on a Flaming-Brown electrode puller (Sutter Instrument Co., Novato, CA, USA) and filled with 3 $mol\,l^{-1}$ KCl. Their resistance measured in saline was 100–140 MΩ. Electrical activity was detected by an Axoclamp 2B amplifier

(Axon Instruments, Burlingame, CA, USA) operating in current clamp mode, connected to a digitizer (Digidata 3200, Axon Instruments) and a laboratory computer. Large (15 μm diameter) dendritic trunks from Type I cells enter the OL medially about 300–350 μm ventrally from its dorsal surface. Therefore, microelectrodes were initially advanced 200 μm into the OL and then in 10 μm steps during attempts to penetrate Type I cells. Neurons used for this study had large (≥50 mV) spikes, responded to both odors and hydrodynamic stimulation, and were recorded from for 20 min or longer, sometimes for as long as 1.5–2 h. Spike data were stored in computer files and were later transferred to CD ROM disks.

Spike counting and data analysis

Data were collected by counting spike numbers during set time intervals following the onset of water or odorant stimuli. The time interval for counting spikes in response to hydrodynamic stimulation was from the onset of water flow until the onset of the odorant pulse. The time interval for counting spikes in response to odorant input was from the onset of the odor pulse until the end of the trailing water pulse. Numerical data were graphed using Microcal Origin software. All data were analyzed by two-tailed, paired *t*-test statistics. In those cases where unequal numbers of trials in the proximal→distal and distal→proximal flow directions were obtained, data from the final one or two trials were ignored as required in order to use paired trials for the statistical analysis.

Results

Fig. 3 summarizes, as reported previously (Mellon, 2005), electrophysiological results obtained from Type I neurons in response to hydrodynamic and odorant stimulation, in this case in response to fluid flow past the lateral antennular flagellum in the proximal→distal direction. In Fig. 3A, short latency spike bursts occurred at the onset of both the water and odorant pulses, and a long latency spike train was generated in response to the odorant. The oscillatory nature of the later spike train is typical for many Type I neurons, although the cellular mechanisms responsible are unknown. The response latency for hydrodynamic stimuli was usually about half that for the odorant stimuli, presumably due not only to faster conduction velocity of mechanoreceptor sensory neurons compared to that of the exceedingly small (≤0.3 μm in diameter) axons of the ORNs (Mellon et al., 1989; Mellon, 1997), but also due to the dead space in the delivery tubes. Fig. 3B compares the response latencies to water onset and to odorant at four different concentrations, while Fig. 3C illustrates a dose–response function for this neuron, confirming its identity as Type I (Mellon and Alones, 1995; Mellon, 1996; Mellon, 2005).

Crayfish flick their antennules in response to novel odors and to mechanical stimulation of the head appendages. In view of the sensitivity of Type I brain neurons to hydrodynamic inputs (Mellon, 2005) it is probable that flicking itself generates substantial hydrodynamic inputs. In *Procambarus* flicking is accomplished by a muscle within the third basal segment of the antennule, inserting upon the most proximal annulus of the lateral flagellum (Mellon, 1997). The entire flagellar structure therefore rotates around this point during a downward flick, and because of the upwardly curved structure of the distal flagellum and its flexibility, the vector of fluid flow around the flagellum during a flick will change in a continuous fashion from the base to the tip. Calling U_{nf} and U_{tf} the velocity components normal and tangent, respectively, to the flagellum at a particular location along its length, analysis relating to fig. 1 in the following paper (Humphrey and Mellon, 2007) shows that $U_{tf}/U_{nf}\approx 0$ along the proximal two-thirds of the flagellum, but that it increases from 0 to 0.84 along the distal one-third; thus, at least along the distal part of the lateral flagellum, downward flicks will generate a predominant flow vector in the P→D direction. Due to the integration of hydrodynamic and odorant stimuli by Type I neurons, it was therefore of interest to determine whether there was a preferred directional sensitivity of these central neurons to fluid flow past the antennular flagellum. We recorded successfully from 23 Type I neurons in 17 isolated head preparations, in most of which we were able to run multiple tests of directional fluid-flow preferences. Fig. 4

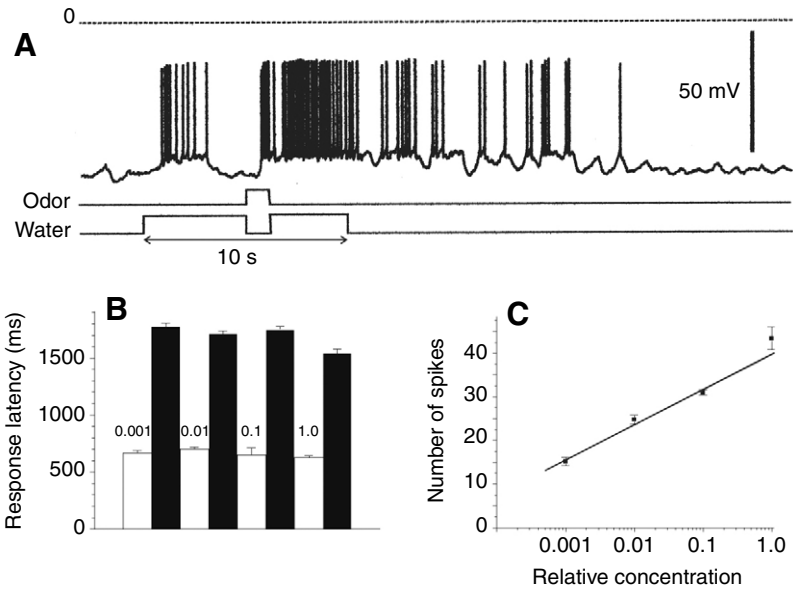

Fig. 3. Hydrodynamic and odorant response characteristics of Type I OL interneurons. (A) Intracellular records from a Type I cell in response to water and odorant pulses (indicated by upward excursions in horizontal lines below the record). The response to the onset of the water pulse was a phasic burst of impulses followed by a shallow hyperpolarization. The response to a brief (1 s) odor pulse consisted of a short hydrodynamic and a much longer spike train, the latter being dose-dependent. The dotted line (marked 0 in this and following figures) indicates the zero potential level. (B) The difference in response latencies between hydrodynamic (open bars) and odorant (filled bars) responses. Numerals next to each pair of bars show relative concentrations of the standard (0.1% w/v) tetramin odorant. Each bar is the mean ± 1 s.e.m. of five responses. (C) Response–intensity function of the neuron represented in A and B. Each data point is the mean ± s.e.m. of five odor presentations at that relative concentration to standard tetramin. The linear fit of the data points is described by the equation $y=8(\log x)+39.6$, $R=0.99$.

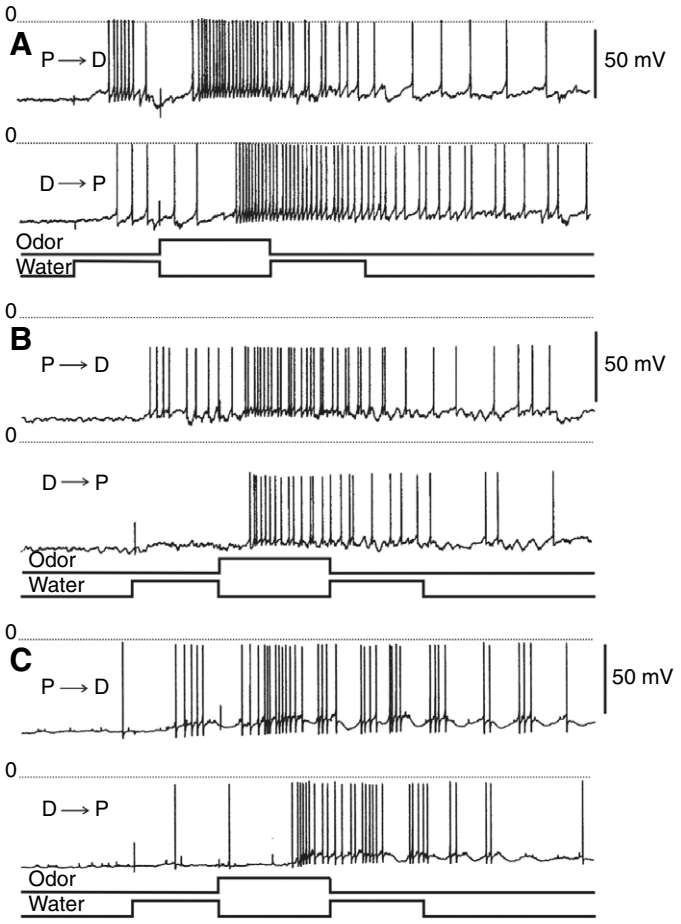

Fig. 4. (A–C) Typical paired records from Type I neurons in three different preparations to water and odorant flow past the antennular flagellum in the (P→D) proximal-to-distal and the (D→P) distal-to-proximal directions. The cells were most sensitive to hydrodynamic movements in the P→D direction, whereas the responses to odorants in the two respective flow directions were not very different from one another (see data in Fig. 5). The hydrodynamic aspects of odorant onset were damped by adaptation to the previous (water onset) stimulus and by the nearly seamless operation of the switching valve. At the start of a standard stimulus sequence, water was suddenly switched on from a no-flow condition; after 3 s odor was seamlessly exchanged for water within the olfactometer during a 4-s period, after which water replaced the odor flow for an additional 3 s. Rates of water flow through the olfactometer with the antennular flagellum in place for the neurons in A and B were, respectively, 15 ml min^{-1} (P→D) and 14.4 ml min^{-1} (D→P), 14.7 ml min^{-1} (P→D) and 14.4 ml min^{-1} (D→P). Odor flow rates for A and B, respectively, were 15.6 ml min^{-1} (P→D) and 14.4 ml min^{-1} (D→P), 18 ml min^{-1} (P→D) and 16.8 ml min^{-1} (D→P). Flow rates of water through the olfactometer in C were 14.4 ml min^{-1} (P→D) and 13.8 ml min^{-1} (D→P). Odor flow rates were 17.4 ml min^{-1} (P→D) and 15 ml min^{-1} (D→P), respectively.

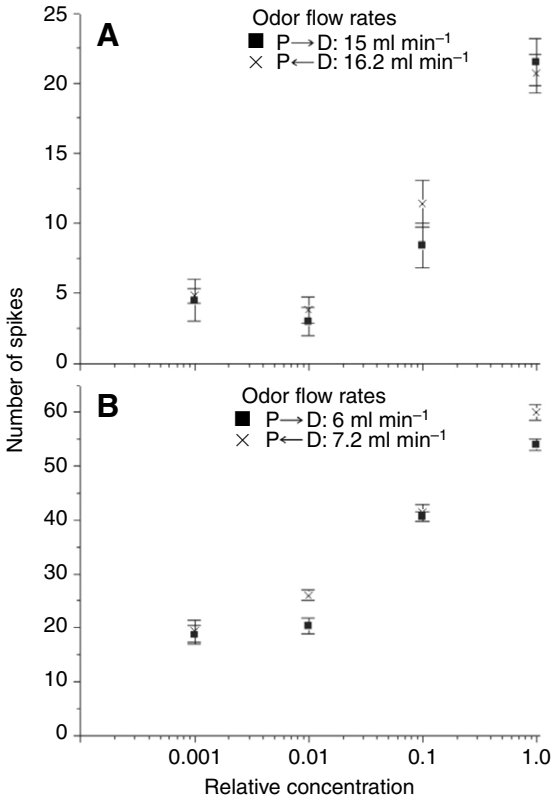

Fig. 5. (A,B) Dose–response relationships for two Type I interneurons to different concentrations of tetramin delivered, respectively, in the P→D flow direction (squares) and the D→P flow direction (crosses). Each point is the mean ± 1 s.e.m. of spike responses to five odorant presentations. Linear fits of the responses of the neuron in A to odorant flows in the two directions are described by the equations (P→D), y=13.4(log$x$)+53.4, R=0.97; (D→P), y=14.8(log$x$)+57.5, R=0.98. Linear fits of the responses of the neuron in B to odorant flows are described by (P→D), y=5.6(log$x$)+16.9, R=0.84, (D→P), y=4.3(log$x$)+16.7, R=0.86. Paired t-test statistics for each of the means in the two plots indicate that, with one marginal exception (A, 0.1 relative concentration), the plots for the respective flow directions in each cell are not significantly different from each other.

shows typical records from three Type I neurons to water and odorant flow in the two directions past the flagellum. Hydrodynamic flow in the P→D direction generates the largest response in terms of spike number and frequency. As has been reported previously (Mellon, 2005) the initial excitatory response is normally followed by a hyperpolarization of the membrane potential and a small post-inhibitory rebound excitation. These initial response phases can interact with the odorant response, enhancing the responses of the interneuron in a non-linear manner when both stimuli are presented simultaneously to the lateral flagellum (Mellon, 2005). In the present cases, separation of the stimuli in time prevented this summation. The latency difference between responses to odorant flowing in the D→P direction compared to flow in the P→D direction is probably the result of the distance of the flagellar tip from the B entrance port, which will be smaller for preparations with longer antennules than for those with shorter antennules. We conclude from these and an additional twelve Type I neurons that the direction of odorant flow past the antennular flagellum made little if any consistent difference in terms of the long-latency response magnitude of these cells. Additionally, Fig. 5 shows quantitative data obtained from two of four Type I cells held for sufficient time to run an extensive dose–response test series. In these two neurons the dose–response curves for the P→D and D→P directions were

nearly superimposeable. For the cell in Fig. 5B, a second test series was performed using a different odorant (Prime Reef™). Dose–response functions for this additional series had a different slope, but again the curves for the two directions were essentially superimposeable (data not shown)

On the other hand, from the records of Fig. 4 it is clear that maximum hydrodynamic responses of these cells to both the onset of the water pulse and odorant pulse occurred when the flow direction was P→D, rather than in the opposite direction. This asymmetry was observed in 11 of the 14 neurons, from which sufficient directional data were available to run statistical tests, as well as in two additional cells in which the recording situation was lost before sufficient trials could be run. Mean data from 12 of the neurons tested are presented in Fig. 6. The overall difference between the mean spike responses of these 12 cells to hydrodynamic flow in the two directions was significant at P=0.00218.

Occasionally we recorded from neurons in the OL that responded only to hydrodynamic stimuli, but had latencies comparable to those of responses to odorants (0.8 s) and a very pronounced directional preference for flow past the antennule in the P→D direction. These neurons showed rapid adaptation to fluid movement in this direction and disadapted slowly over a period of 10–20 s. They were unaffected by fluid movement in the D→P direction. The contribution of these purely hydrodynamic receptive neurons to Type I cell responses, if any, is not known.

Discussion

The data presented in the present study indicate that there is a directional asymmetry in the magnitude of responses in Type I OL interneurons to fluid flow past the lateral antennular flagellum in *Procambarus*, with the preferred flow stimulus being proximal-to-distal. Several different cellular mechanisms could account for this asymmetry, including: (i) the restriction of Type I inputs to unidirectional mechanoreceptor sensilla on the flagellum, or to chordotonal proprioceptors within the flagellum, responding to fluid flow only in the preferred direction; (ii) suppression through pre- or postsynaptic inhibition of other inputs to Type I neurons from uni- or bidirectional mechanoreceptor sensilla responding to fluid flow in the null direction; (iii) selective facilitation or gating of synapses on Type I dendrites by mechanoreceptors excited by fluid movements in the preferred direction; or (iv) a combination of these or other mechanisms. Delayed postsynaptic inhibition is in fact a characteristic of responses to hydrodynamic stimulation in Type I neurons (Mellon, 2005). However, our present studies have not revealed a more robust inhibition following fluid movements past the antennule in the null direction. If anything, the reverse was found to be true, with the hyperpolarization that followed the initial excitatory response to water onset being larger in the preferred rather than the null direction. Furthermore, the hyperpolarizing phase of the response always has roughly half again the latency of the initial excitation and, therefore, it would not be expected to have an overriding influence on initial response magnitude. In the absence of evidence to the contrary, the most probable mechanism to explain the response asymmetry in the Type I cells is their targeting by directionally sensitive mechanoreceptor neurons in the flagellum. Such neurons are well known from studies of bidirectional mechanoreceptive sensilla on other anatomical regions of *Procambarus* (Mellon, 1963; Wiese, 1976; Wiese et al., 1976), although the morphological type characteristic of bimodally sensitive sensilla on the uropods, telson and branchiostegites supplied by these neurons has not yet been specifically identified on the crayfish antennules. Wiese et al. (Wiese et al., 1976) recorded intracellularly from first- and higher order interneurons within the sixth abdominal ganglion of *P. clarkii* and examined integration of hydrodynamic inputs from bidirectional mechanoreceptive sensilla on the telson. They found that many interneurons received excitatory input from one specific class of directionally sensitive mechanoreceptor neurons, responding to sensilla movement either in the rostral or the caudal direction, but not to both. Some interneurons responding to fluid motions in one direction were in fact inhibited by motion in the opposite direction, thereby displaying an enhanced response specificity to direction of water flow past the sensillum (Wiese et al., 1976). More recently, Tautz and Plummer recorded from the caudal photoreceptor neuron and a local directionally sensitive non-spiking neuron in the sixth abdominal ganglion of the crayfish *Orconectes limosus* (Tautz and Plummer, 1994). In brief, they found that the local directionally sensitive neuron and its postsynaptic target, the caudal photoreceptor, both had strong preferences for the direction of the flow of a stream of saline over the tail fan of the animal. Thus, both the underlying sensory apparatus as well as the concept of central integration of directionally specific hydrodynamic information in the crayfish have precedent in experimental observation.

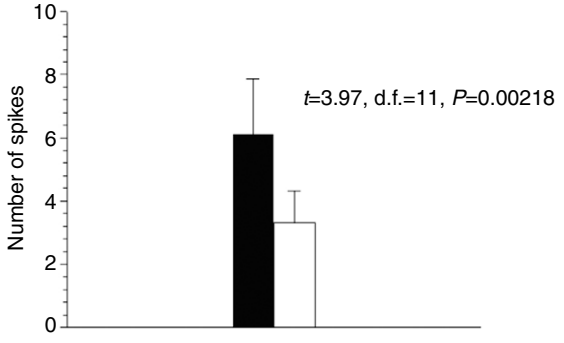

Fig. 6. Bar graph illustrating differences in the spike responses of 12 Type I neurons following the onset of water flowing past the antennular flagellum in the P→D direction (filled bar) and in the D→P direction (open bar). Data represent the mean number of spikes following stimulus presentations (± 1 s.e.m.); the number of stimulus presentation pairs from which the means were calculated for each preparation was between 4 and 27. Statistical significance of the differences between the means is P<0.0025. The flow rate in the D→P direction in each experiment was normalized to the P→D flow rate. The mean value for flow rate obtained across all experiments was 1.12±0.06 (± s.e.m.).

Type I deutocerebral interneurons in *Procambarus* are more sensitive to fluid movement past the flagellum in the proximal-to-distal than in the opposite direction. As discussed in relation to fig. 10 in the following paper (Humphrey and Mellon, 2007), the proximal-to-distal flow in the tube experiment corresponds

fairly closely, both in magnitude and direction, to the flow past a significant length of a downward-flicking flagellum, especially for putative mechanoreceptor sensilla along the distal one-third of the flagellum. Furthermore, along the proximal third of a flagellum in the reversing olfactometer, computations of the drag forces and torques exerted on the mechanoreceptor sensilla compare well with the corresponding values for a flicking flagellum [see discussion of fig. 12 and table 1 in Humphrey and Mellon (Humphrey and Mellon, 2007)]. We can thus safely rule out the possibility that the olfactometer may have generated artifactual neural responses, while noting that the experimental conditions to which the antennules were exposed within it were clearly not beyond the physiological range normally experienced by the animal. Because of the close correspondence in flow patterns, and the associated drag forces and torques acting on the mechanoreceptor sensilla, we conclude that it is probable that the receptors for hydrodynamic stimulation generating the responses in our experiments respond to water movements encountered during a normal flick.

The most numerous setal type found on both the medial and lateral antennular flagella in *Procambarus* are those we refer to as beaked hairs (Fig. 1B). They range from 50 to 150 μm in length and are characterized by a hook or beak at their tip. They are present on most annuli of the lateral flagellum, arranged around the anterior circumference of each annulus, especially on the medial, lateral, dorso-medial and dorso-lateral aspects. Recent unpublished observations in our laboratory have provided preliminary evidence that the beaked hairs have associated sensory neurons that respond phasically to tactile stimulation, although details concerning their preferred plane of movement and sensitivity to water currents are currently lacking. Because of the prevalence of beaked hairs on the lateral flagellum and their putative mechanosensory function, however, our modeling and theoretical treatment of hydrodynamic events along the flagellum have focussed on this type of seta.

As determined theoretically in the following paper (Humphrey and Mellon, 2007), the beaked hairs on the ventral aspects of the flagellum will be subjected to maximum torque toward the flagellum during a downward flick, whereas those on the dorsal aspects will be subject to maximum torque during the return stroke. Beaked hairs projecting from the medial and lateral surface of the flagellum will be subject to similar drag forces in both the down stroke and return stroke of a flick, although the torque experienced will be in opposite directions in the two respective cases. These asymmetries in drag and torque experienced by different populations of beaked hairs during a flick cycle are similar in magnitude and direction to those experienced by setae in the reversible-flow olfactometer (Humphrey and Mellon, 2007), although it has not yet been established that they are specifically causative to the asymmetry in the responses of Type I neurons to fluid flow in the P-D and D-P directions. Finally, as discussed in the accompanying paper, initial observations using the atomic force microscope (C. Jennings and E. Berger, unpublished) indicate that beaked hairs are essentially inflexible cylinders that can be deflected from the resting position without bending. This finding has important theoretical implications in modeling hydrodynamic flow fields around these structures.

Enhancement of chemoreceptor function by flicking

Koehl and her colleagues have proposed the hypothesis that downward flicking of crustacean antennules encourages regeneration of the boundary layer around the aesthetasc sensilla (Koehl et al., 2002; Koehl, 2005), thereby periodically capturing novel odorant-bearing water samples. In the spiny lobster and other marine crustaceans the aesthetascs are grouped together in dense aggregations near the distal end of the lateral flagellum. It has been argued previously that downward flicking splays out this aggregation, thereby flushing the entrapped water around the sensilla (Snow, 1973; Schmitt and Ache, 1979). Furthermore, because the diameters of the aesthetascs are small (10–20 μm) they tend to be embedded in the flagellum boundary layer, and flicking presumably assists in regenerating this layer, thereby allowing access of the aesthetasc cuticle to new water samples. Although the aggregations of aesthetascs on crayfish antennules are relatively sparse compared to, for example the spiny lobster (Tierney at al., 1986; Mellon et al., 1989; Laverack, 1964; Ghiradella et al., 1968; Grünert and Ache, 1988), the minute size of the individual aesthetascs suggests that they too will be embedded in the flagellum boundary layer during a downward flick. Therefore, as in the spiny lobster, the argument that flicking disrupts this layer, permitting exposure of the crayfish aesthetasc surface to new water samples, has merit, especially when considering the wake vortices generated by the flagellum during the recovery phase following a downward flick (Humphrey and Mellon, 2007).

Direct electrophysiological measurements have shown that flicking temporally enhances the spiking responses of spiny lobster ORNs to odorant stimuli (Price and Ache, 1977; Schmitt and Ache, 1979). Schmitt and Ache suggest that this improves detection of just-threshold concentrations of water-borne odorants (Schmitt and Ache, 1979). In theory this process could itself be amplified by combining input from temporally enhanced ORN spiking with hydrodynamic inputs generated during a downward flick to bimodal first order interneurons in the deutocerebrum, such as Type I cells or their equivalent in the lobster OL (Schmidt and Ache, 1996). Single-unit electrophysiological observations of crayfish ORN responses to odors have not yet been obtained, and future work in this area will be required to understand whether flicking enhances activity at the periphery. Additional experiments with Type I neurons during passive and active flicking of the lateral filaments in the presence of otherwise static, homogeneous fluid environments must, however, be carried out to determine how hydrodynamic and olfactory sensory modalities are combined centrally to improve odorant detection during more realistic stimulus regimes than those of our current experimental procedures. Flicking is an active process and corollary discharges from central neurons that trigger this behavior could in theory play an important role in odorant detection by facilitating synaptic transfer of information from the periphery to central neurons.

Flicking has apparently evolved as a behavior that enhances chemical receptivity by disruption of the boundary layer of water surrounding aesthetascs and possibly other types of sensilla, and by entrapment of newly sampled water. This enhanced information is then incremented centrally by combining it with hydrodynamic input generated through the

same behavior. In light of the cumulative findings of this study, and in view of the confirmatory theoretical findings discussed in the following paper (Humphrey and Mellon, 2007), it seems reasonable to postulate that critically timed enhancement of synaptic transfer of this peripheral input could be a useful additional step in the detection of odors under marginal situations.

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