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Response properties of crayfish antennules to hydrodynamic stimuli: functional differences in the lateral and medial flagella

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SUMMARY

Antennules have been reported to influence localization of distant food odors, sex discrimination, and agonistic and social behaviors of decapod crustaceans. Although olfaction by the antennules is largely recognized, information on the sensitivity of antennules to hydrodynamic stimuli has been scant. In red swamp crayfish *Procambarus clarkii* antennules, mechanosensory setae outnumber the chemosensory setae. We studied the mechanosensitivity of crayfish antennules by recording neural activities from isolated antennules in response to sinusoidal dipole stimuli. Both the lateral and the medial flagellum of the antennules responded to hydrodynamic stimuli, although the medial flagellum showed more sensitivity at frequencies higher than 60 Hz. The most dominant setae present on the stimulated site were the simple setal type. Although both lateral and medial flagella are capable of detecting chemical and hydrodynamic cues, results from neural responses, morphological observations and antennular behavior observations indicate that the lateral flagellum of *P. clarkii* functions as an olfactory organ whereas the medial flagellum complements as a hydrodynamic receptor. It appears that in crayfish antennular sensory processing, crayfish simultaneously use chemical and hydrodynamic information. We have compared our data with the threshold of fish lateral line to the same stimuli and we discuss probable similarities in response properties.

Key words: Crustacea, antennules, mechanoreception, flicking, fish lateral line.

INTRODUCTION

In decapod crustaceans, the antennule has been reported as an olfactory organ with an important role in food searching, mating and agonistic behavior. The antennule is biramous, with a lateral and a medial flagellum. The lateral flagellum, which bears chemoreceptors referred to as aesthetascs, functions as a detector of food odors (Laverack, 1988; Atema, 1995; Giri and Dunham, 1999; Steullet et al., 2001; Steullet et al., 2002), sex odors (Christofferson, 1972; Ameyaw-Akumfi and Hazlett, 1975; Gleeson, 1982; Bamber and Naylor, 1996; Kamio et al., 2005) and social odors (Obermeier and Schmitz, 2003). Flicking the antennules by crustaceans is believed to enhance the detection of chemical cues by aesthetascs (Snow, 1973; Schmitt and Ache, 1979; Koehl et al., 2001). In the crayfish *Procambarus clarkii*, the antennular lateral flagellum possesses aesthetascs and associated guard and companion setae on its ventral surface. Each aesthetasc sensillum contains the distal dendrites of about 170 olfactory receptor neurons, the axons of which course through the antennular nerve to the olfactory lobe (Mellon et al., 1989). Aesthetascs play an important role in intraspecific communication (Horner et al., 2008). Behavioral experiments after ablation of the entire flagellum in crayfish demonstrated that the lateral flagellum also mediates the perception of food odors (Dunham et al., 1997; Giri and Dunham, 1999) and sex discrimination (Ameyaw-Akumfi and Hazlett, 1975; Dunham and Oh, 1992; Giri and Dunham, 2000), while electrophysiological recordings from crayfish deutocerebrum interneurons showed responses when the lateral flagellum was stimulated with food extracts (Mellon and Humphrey, 2007).

By contrast, the role of the medial flagellum in crayfish antennular sensory processing is still unclear. Previous studies on the role of the medial flagellum on the sensory process yielded inconsistent and contradictory results. Ameyaw-Akumfi observed that the ablation of both flagella did not affect the ability of *P. clarkii* to perceive food odors (Ameyaw-Akumfi, 1977). However, Dunham et al. (Dunham et al., 1997) reported that the medial flagellum could mediate a response to sucrose, whereas Giri and Dunham (Giri and Dunham, 1999) demonstrated that a large number of *P. clarkii* with only the medial flagella present were unable to locate long-distance food sources. On the role of the medial flagellum in the detection of sex and social odors, Ameyaw-Akumfi and Hazlett (Ameyaw-Akumfi and Hazlett, 1975) reported that the medial flagella of the antennules are the site of sex discrimination in *P. clarkii*. Although Dunham and Oh (Dunham and Oh, 1992) reported that *P. clarkii* performed sex discrimination even with only one lateral and one medial flagellum, this discrimination ability was reduced for those animals with only the two lateral flagella or only the two medial flagella. Giri and Dunham (Giri and Dunham, 2000) reported that both the lateral and medial flagella in female *P. clarkii* were equally useful in the detection of male conspecifics. On the contrary, ablation tests by Tierney et al. (Tierney et al., 1984) showed that the lateral flagellum mediates pheromone reception in *Orconectes propinquus*. Horner et al. (Horner et al., 2008) demonstrated that aesthetascs in the lateral flagellum mediate communication between *P. clarkii* conspecifics.

Mechanosensory setae are present on the thorax, chelae, tailfan and antennae in crayfish (Mellon, 1963; Tautz and Sandeman, 1980; Tautz et al., 1981; Bender et al., 1984; Douglass and Wilkens, 1998). On the antennules, at least five non-aesthetasc setal types have been reported to be mechanosensitive: procumbent plumose setae, standing plumose setae, smooth conate setae, long simple setae and medium simple setae (Chichibu et al., 1978a; Chichibu et al., 1978b; Tautz et al., 1981; Bender et al., 1984; Mellon and

Christison-Lagay, 2008). Most prominent on the distal half of crayfish antennules are medium and long simple setae that project from the dorsal side of the lateral flagellum and on the dorsal, ventral and lateral sides of the medial flagellum. Medium simple setae had been previously identified as S-type hairs and beaked hairs by Chichibu et al. (Chichibu et al., 1978b) and Mellon and Humphrey (Mellon and Humphrey, 2007), respectively, and had been reported to be mechanosensory. Chichibu et al. (Chichibu et al., 1978a) studied the response of this seta to mechanical stimulation by displacement using a glass capillary. Mellon and Humphrey (Mellon and Humphrey, 2007) and Humphrey and Mellon (Humphrey and Mellon, 2007) reported that this setal type responded differently to opposite water flows and correlated this asymmetry to the varying drag force magnitudes acting on the mechanoreceptors during the upward and downward flick.

In this work, we studied the structure and function of setae on the antennules of the crayfish *P. clarkii*. First, we studied the morphology of setae on the dorsal section of the distal half of their antennules. Then, we investigated the sensitivity of crayfish lateral and medial antennular flagella to water particle motion in the form of a sinusoidal dipole stimulus. This method might not imitate the actual noisy aquatic environment, but sinusoidal driven dipoles offer a good starting point for the study of mechanosensitivity of crayfish antennules in view of the scarcity of information regarding this subject. We also examined antennule behavioral responses to stimuli. Based on setal morphology and on physiological and behavioral responses to hydrodynamic stimuli, we suggest that although both lateral and medial flagella are capable of detecting chemical and hydrodynamic cues, the lateral flagellum primarily functions as an olfactory organ, while the medial flagellum complements the sensory process by acting as a hydrodynamic detector. It appears that in antennular sensory processing, *P. clarkii* uses both the chemical and hydrodynamic signals produced by a potential aggressor, mate, predator or prey.

MATERIALS AND METHODS

Test animal

Adult red swamp crayfish *Procambarus clarkii* Girard, 35–40 mm in carapace length, were obtained from a local supplier in Kagoshima, Japan. Animals were held in tanks with aerated water at 20–25°C until used for experiments. Holding tanks were supplied with *Elodea* and animals were fed twice a week with crayfish pellets (Japan Pet Drugs Company, Katsushika, Tokyo, Japan) and were kept under a light:dark regime of 12 h:12 h.

SEM preparation

Antennules from six newly molted crayfish were cut and fixed in 2.5% glutaraldehyde in PBS solution (0.1 mmol l^{-1}, pH 7.2). After 24 h, fixed samples were rinsed in PBS, dehydrated in an ethanol series, dried using a t-BuOH freeze dryer (VFD-21S, Vacuum Device Company, Uchihara, Ibaragi, Japan) and gold coated (Magnetron spatter MSP-10, Vacuum Device Inc.). Scanning electron microscope (Hitachi S-4100H, Chiyoda, Tokyo, Japan) observations were performed over the lateral and medial flagella.

Hydrodynamic stimulus

The crayfish antennule was stimulated as described by Watanabe and Anraku (Watanabe and Anraku, 2007). A unidirectional and sinusoidal dipole source was generated by a small (4.5 mm diameter) plastic sphere that was attached to an oscillator (Akashi Corporation, Zama, Kanagawa, Japan) by a stainless steel shaft (15 cm long, 1 mm diameter). The shaft was mounted perpendicular to the flagellum, with the sphere placed 4 mm from the dorsal section of the flagellum's distal half. The oscillator was driven by a function synthesizer (NF Electronic Instruments, Kohoku, Yokohama, Japan) controlled by a computer (NEC Valuestar NX VE26/4, NEC Corp., Minato, Tokyo, Japan). Sinusoidal signals were digitized with an A/D converter (PowerLab 4/20, ADInstruments, Milford, MA, USA) and stored in a computer. Tested frequencies ranged from 5–200 Hz at 10 Hz increments, each with different amplitudes produced by different voltages. Some fibers were tested at 20–30 Hz increments to enable sampling of higher frequencies because action potentials showed signs of early loss. For the same reason, fibers were tested only once in each amplitude. Threshold determination was aided by visual and audio outputs, and whenever necessary a fiber was tested twice in a particular amplitude. In each frequency, at least five lower and higher amplitudes were tested to ensure the accuracy of threshold determination. Stimulus amplitude was measured prior to the experiment by viewing the transducer shaft movements from the side with a light microscope. The intensity of the stimulus applied to the antennule was computed in terms of the root mean square (r.m.s.) of water particle velocity following the method of Harris and van Bergeijk (Harris and van Bergeijk, 1962). Displacement amplitudes of the sphere used in the test ranged between 0.03 and 23.09 μm r.m.s.

Electrophysiological recording

Crayfish were prepared for recording by immersing them in chilled water for 15 min. The antennule was cut at the base where the lateral and medial flagella bifurcate. This removed the influence of a chordotonal organ present on the basal segments of the antennular ischiopodite (Kouyama and Shimozawa, 1982). The cuticle in at least three segments at the proximal end of the isolated flagellum was removed and the nerves desheathed and exposed. The isolated flagellum was suspended in a Plexiglas container (10 cm× 10 cm×10 cm) containing chilled and oxygenated van Harreveld saline solution (van Harreveld, 1936). The pH of the solution was 7.8 and the temperature of the saline within the recording chamber ranged from 12–14°C at the start to 18–20°C after the termination of recording. Exposed nerves were sucked into a small (40–70 μm inner diameter) glass electrode, initially filled with van Harreveld saline solution, using a threaded suction pump (A-M Systems, Carlsborg, WA, USA). Nerve fibers were tested for hydrodynamic sensitivity by applying small jet streams to the flagellum or slightly touching setae, and only those fibers that showed responses were used in the actual test. In the experiment, pre-stimulus neural activity was recorded for 20 s, after which the stimulus was applied for at least 20 s. Electrical responses were amplified (Nihon Kohden, Shinjuku, Tokyo, Japan) and digitized in synchrony with sinusoidal signals using an A/D converter. A speaker was used to listen to the neural activity. Data were monitored and stored in a computer.

Data analysis

Many of the recordings were either dual or multiunit recordings. Discrimination of a single unit recording was performed using the module software in Chart (Spike Histogram Discriminator, ADInstruments). Data were analyzed using the same software by counting the number of spikes during pre-stimulus and following the onset of the water stimulus. Fibers were considered responsive to the sinusoidal stimulus if discharge rates obtained during stimulation were significantly higher from those during pre-stimulus (two-tailed Student's *t*-test). The minimum threshold to water particle motion in each frequency was determined as the lowest amplitude that produced a significant increase in neural activity.

To analyze phase locking of spikes to the sine wave, each sinusoidal wave stimulus was divided into 36° phases by adjusting the bin size according to the stimulus frequency used. The number of spikes that occurred in each phase of the sinusoidal curve was counted using the Interspike Interval Histogram (ADInstruments). Using circular data statistics software (Oriana, Kovach Computing Services, Anglesey, Wales, UK), the Rayleigh test was performed to determine whether measures of phase locking were statistically significant. A *P*-value of less than 0.05 indicated that spikes occurred in a specific phase of the wave stimulus, whereas a value of more than 0.05 indicated that spikes were randomly distributed during a sinusoidal wave cycle.

Recording with ablated setae

To ensure that recordings were in response to the deflection of the seta and to remove the effect of plumose setae, separate recordings were performed on eight antennules with ablated setae. In the first test, all the setae (except the aesthetascs) were cut or shaved under the microscope. In a second test, only the procumbent and standing plumose setae were removed, either by shaving and/or by cutting a large part of the proximal half in which most of the procumbent and standing plumose setae are located. Both flagellar conditions were similarly stimulated with the sinusoidal dipole and responses were measured.

Antennular activity examination

To examine antennular behavior, three groups of crayfish – intact flagella, entire lateral flagella ablated and entire medial flagella ablated – were individually placed in a 2 l tank filled with aged tap water. The lateral or the medial flagella were cut where both flagella bifurcate. Ten animals were used in each group. Following ablation, crayfish were returned to their individual containers, where they remained for one week prior to testing. Under red illumination, antennular activity was observed for 30 s, after which the animal was stimulated by applying jet streams of artificial freshwater (AFW) at the anterior portion of the animal using a pipette. After 30 min, the same animal was stimulated by jet streams of food extracts (2.5 g food pellets in 50 ml AFW). After stimulation, flicking frequency and behavior were recorded. To test the effect of ablation on antennular flicking, the Mann–Whitney *U*-test was used to compare flicking rates between intact and medial flagellum-ablated individuals. To test significant differences among flicking rates when the crayfish was at rest and during non-odorant and odorant stimulations, the non-parametric Friedman test followed by a Wilcoxon signed-rank test were performed.

RESULTS

Morphological characteristics

The *P. clarkii* antennule is biramous with a lateral and a medial flagellum (Fig. 1A). The lateral flagellum is slightly flattened at the proximal half and the distal half is projected forwards with a slight angle. The medial flagellum is a tapered cylinder and is pointed fronto-laterally within the horizontal cephalo-anal plane. The lateral flagellum is slightly shorter (mean±s.e.m., 19.98±0.29 mm; *N*=90) than the medial flagellum (20.3±0.28 mm). The aesthetascs (Fig. 1B), characterized by their blunt tip, were present on the ventral aspect of the distal half of the lateral flagellum. The most distal segments had three to five aesthetascs occurring in two rows, whereas the segments near the middle of the flagellum had a single row with one to three aesthetascs. Flanking the aesthetascs were the larger guard hairs and, occasionally, the smaller companion cells. Non-aesthetasc setae include at least six types. On the stimulated area, i.e. the dorsal section on the distal half of crayfish antennules, medium simple setae were the most numerous setal type, with occasional occurrence of long simple setae. Medium simple setae possess a single annulation, range in length from 60–90 μm and project at an angle of approximately 45–90° from the flagellar surface (Fig. 1C). The number of simple setae in each segment on the distal half of the flagella was not consistent but there were about two on the dorsal section of the lateral flagellum (Fig. 1B). On the medial flagellum, each segment had about two simple setae on the dorsal section, and four on the ventral and ventro-lateral sections (Fig. 1D). Located on the proximal half of both flagella are the procumbent plumose setae, the smooth conate setae, the standing plumose setae and the short simple setae. The procumbent plumose setae were both the most numerous and the largest setae on the surface of the crayfish antennules (Fig. 1E). These setae lay flat along the surface of the flagella with a length that ranged from 130-420 μm. They originated from one segment and bridged to the next distal segment. The relative frequency of major setal types in each

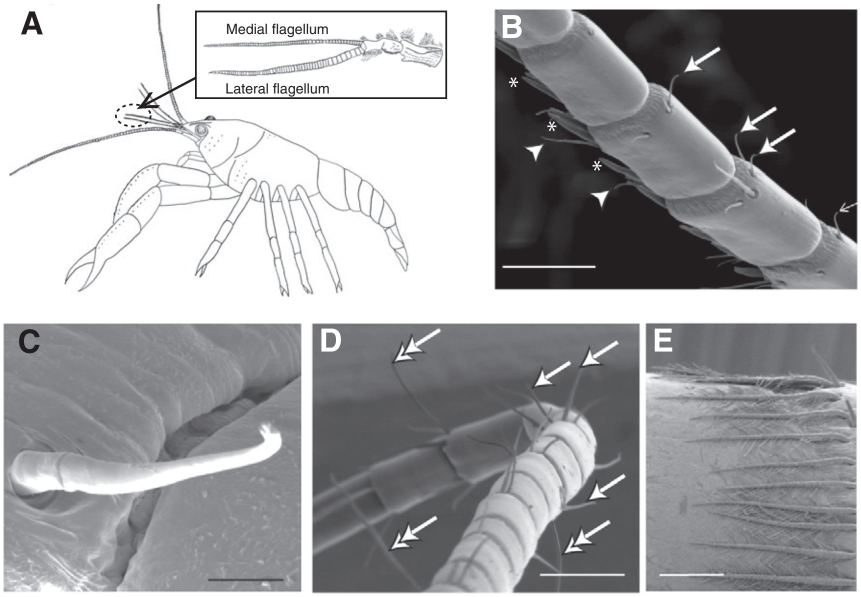

Fig. 1. (A) The biramous antennules of *Procambarus clarkii* showing the lateral and medial flagella. (B) The distal half of the lateral flagellum showing the aesthetascs (asterisks) and the guard setae (arrowheads) on the ventral section, and the medium simple setae (arrows) on the dorsal section (scale bar, 150 μm). (C) Scanning electron micrograph of a medium simple seta on the antennule (scale bar, 15 μm). (D) The distal half of the medial flagellum showing the medium (arrows) and long (double arrows) simple setae on the ventral, dorsal and lateral sides (scale bar, 150 μm). (E) Procumbent plumose setae on the proximal half of both flagella (scale bar, 150 μm).

segment of crayfish antennules is shown in Fig. 2. In the lateral flagellum, the distal half possessed more aesthetasc, guard and companion setae. In each segment of the distal half of the lateral and the medial flagella, medium simple setae comprised about 10–46% and 40–92% of the total setal count, respectively. A clear grouping of major setae in the crayfish antennules is indicated; the aesthetascs and associated setae are present at the ventral side of the lateral flagellum's distal half, procumbent plumose setae occupy the proximal half, whereas medium simple setae are dominant in the distal half of both the lateral and medial flagella.

Antennule response to sinusoidal stimulus

Neural activity was recorded in 194 fibers. Mean spontaneous discharge activity was 2.7±1.7 spikes per second. In many cases, spike height decreased with time and the frequency of ongoing spike activity was reduced, but sensitivity to the sinusoidal stimulus remained. Three types of response to sinusoidal stimulus were recorded from the isolated antennules (Fig. 3). The first type responded with an increased discharge rate with sufficient stimulus amplitude. The second type decreased discharge rates when stimulated at higher frequencies, usually at frequencies higher than 200 Hz. The third type yielded no response to sinusoidal stimulus, although this type generally had a decrease in spike activity when stimulated at high frequencies of 200 Hz or more. The first and second types of response were tonic, characterized by a rapid increase or decrease in discharge rate at the onset of stimulation that lasted until the termination of the stimulus. All fibers that exhibited an increase in the number of spikes during stimulation showed phase locking (Rayleigh test, $P<0.05$), the degree of which increased with increasing stimulus amplitude. Table 1 shows the occurrence of spikes along the sinusoidal curve when stimulated with a displacement that was double the threshold in a given stimulus frequency. At any given frequency, spikes occurred close to the 360°/0° phase of the sinusoidal curve. The probability that the spike would appear in the same phase was highest at 200 Hz.

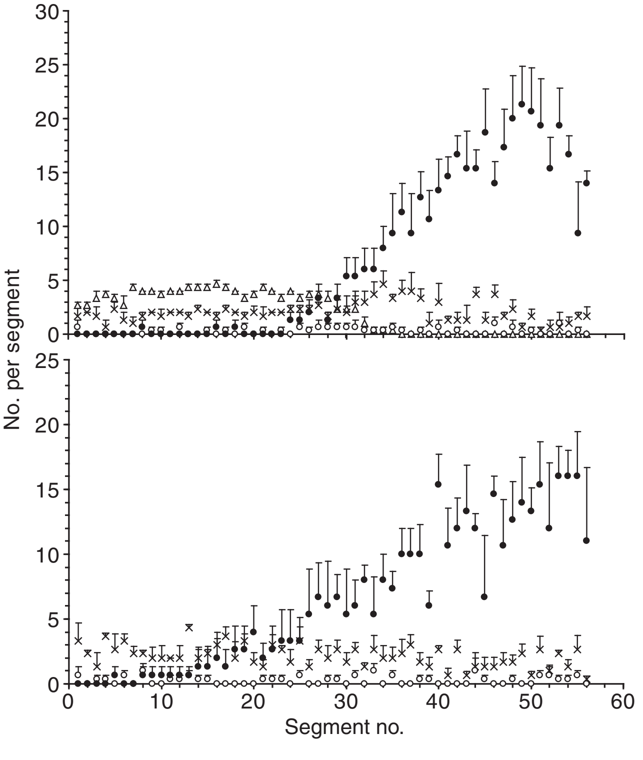

Fig. 2. Relative density and distribution of medium simple setae (x), aesthetascs (triangles), long simple setae (open circles) and procumbent plumose setae (filled circles) in each segment of the lateral (top) and medial (bottom) flagella of crayfish antennules. These values (mean±s.e.m.) were from the first 56 segments (indicated on the abscissa axis) of three animals, with segment no. 1 representing the distal tip of the antennule.

The threshold of the lateral and medial flagella to water particle velocity is shown in Fig. 4A. In both flagella, antennular threshold to water particle velocity decreased from 5 to 50 Hz, then gradually increased to 200 Hz. The lowest mean threshold to water velocity was observed at 50 Hz stimulation where the lateral and medial flagella had mean thresholds 35 and 38 μms^{-1} r.m.s., respectively. Mean thresholds of both flagella when stimulated at 5–60 Hz were not significantly different (*t*-test, $P>0.05$). Beyond 60 Hz, the medial flagellum showed more sensitivity to the velocity of water particle motion (*t*-test, $P<0.05$).

Response of antennules with ablated setae

Sinusoidal stimulation of seta-less lateral and medial flagella did not induce any response. Spike activity remained unchanged upon stimulation. When the seta-less flagella were stimulated at high frequencies such as 1000 Hz, neural activity remained unchanged. In recordings with intact flagella, spike rate would normally decrease when stimulated at frequencies higher than 300 Hz. The absence of response in seta-less antennules and during stimulation at high frequencies suggests that responses resulted from the deflection of the setal shaft and not from chordotonal organs.

By contrast, crayfish antennules without procumbent and standing plumose setae continued to respond to sinusoidal stimuli, and threshold values in both lateral and medial flagella were similar to those recorded from intact flagella (Fig. 4B,C).

Antennular activity

Antennular flicking in crayfish involved the downward stroke of the lateral flagellum and its rapid return to the original position. In *P. clarkii*, the distal half of the lateral flagellum is arched upwards

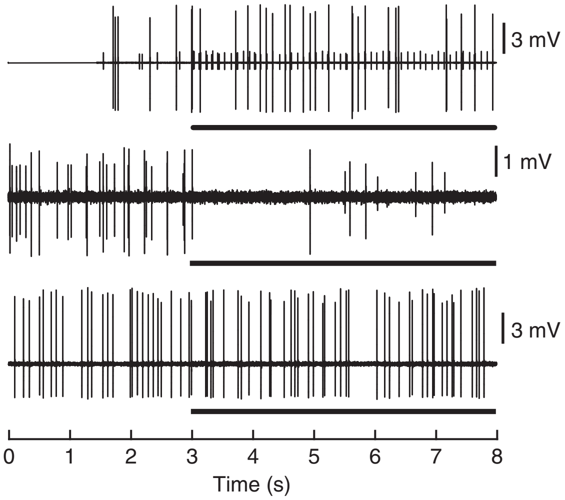

Fig. 3. Type of crayfish antennular response to sinusoidal stimulation. Stimulation induced an increase (top), decrease (middle) or no change (bottom) in the number of neural discharges. The first and second types exhibited tonic response to the stimulus.

Table 1. Phase-locking properties of crayfish antennules when sinusoidally stimulated at different frequencies

Stimulus frequency (Hz)	Mean vector (μ)*		Length of mean vector (r)†		95% confidence interval (±) for μ				Rayleigh test (P)	
					Min.		Max.			
	Lateral	Medial	Lateral	Medial	Lateral	Medial	Lateral	Medial	Lateral	Medial
10	2.6	6.2	0.45	0.47	354.5	356.9	10.7	15.5	<0.01	<0.01
40	359.4	1.0	0.37	0.48	349.0	355.1	9.8	6.9	<0.01	<0.01
50	0.5	359.6	0.70	0.66	356.8	355.5	4.2	3.8	<0.01	<0.01
70	359.9	11.3	0.66	0.41	355.3	3.0	4.4	19.6	<0.01	<0.01
100	359.2	1.9	0.62	0.58	353.7	357.0	4.8	6.8	<0.01	<0.01
200	0.9	1.1	0.77	0.70	357.7	357.3	4.1	4.8	<0.01	<0.01

*The vector μ is the mean phase along the sinusoidal curve that spikes occurred during stimulation.

†The length of the mean vector (r) indicates the probability that the spike will occur in the same phase of the sinusoidal curve.

such that, during flicking, the ventrally located aesthetascs are positioned to sample the environment to the anterior of the head. The medial flagella were not flicked; they might be moved slowly, but not in a manner similar to the rapid downward and upward strokes of the lateral flagellum during flicking. In addition to flicking, stimulation by water jet streams and food extracts induced antennular depression in crayfish. This behavior involved a pair of flagella or the entire antennules being pressed down at an angle approximately 30–90° towards the mouth area (Fig. 5A). The flagella remained depressed for a few seconds (usually 1–3 s), after which the lateral flagellum was flicked again or both flagella were returned to normal position.

The antennular flicking rates in individuals with intact and lateral-only flagella are shown in Fig. 5B. When at rest, flicking in both groups was infrequent, at a rate of 0–0.25 flicks per second. The following stimuli initiated or increased the frequency of flicking: applying jet streams to the cephalothorax; tapping the

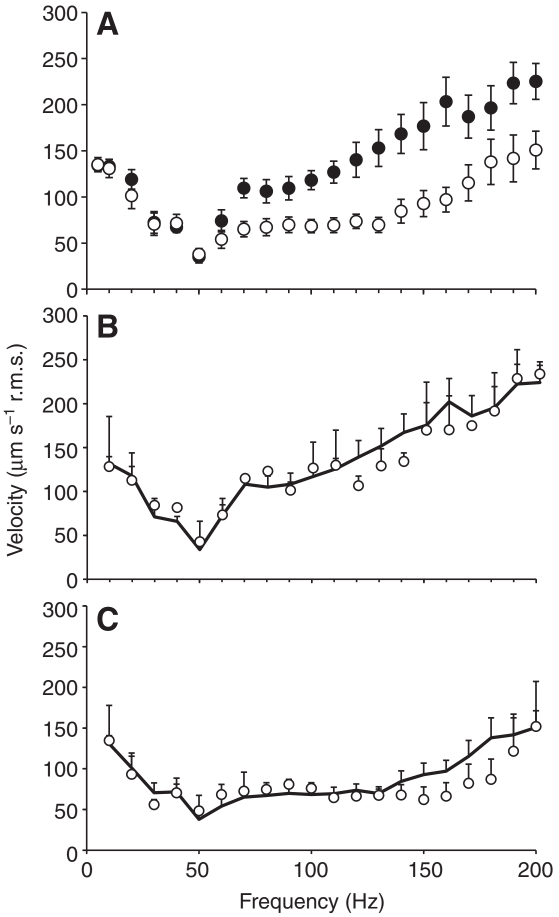

Fig. 4. (A) Threshold of crayfish lateral (filled circles) and medial (open circles) antennular flagella to the velocity of water particle motion at different stimulus frequencies. At each frequency, the number of replicates (N) ranged from 31 to 66. Threshold of lateral (B) and medial (C) flagella with intact setae (solid line) or with procumbent and standing plumose setae removed (open circles) to water velocity. All values represent mean±s.e.m.

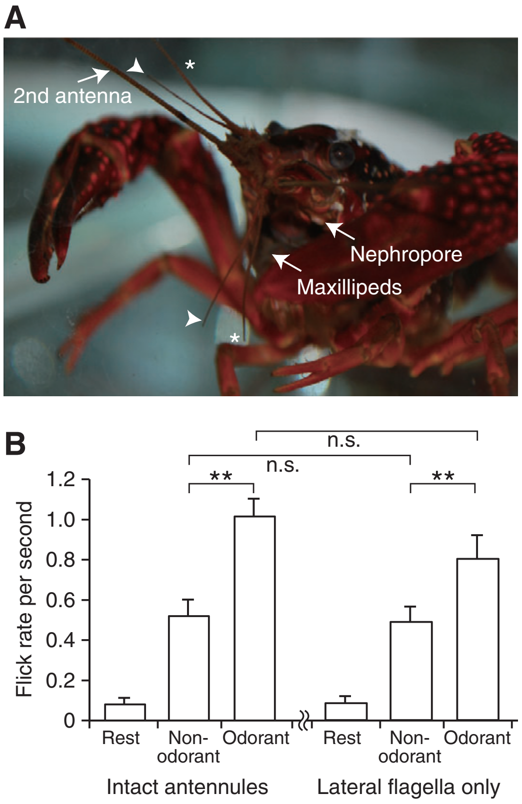

Fig. 5. (A) Depression of the antennules in *P. clarkii*. A pair of flagella or the entire antennules are lowered into the mouth area while crayfish beats the maxillipeds. Lateral and medial flagella are indicated by asterisks and arrowheads, respectively. (B) Flicking rates (mean±s.e.m.) of *P. clarkii* with intact antennules or with ablated medial flagella, when at rest and during stimulation with non-odorant and odorant jet streams. Asterisks indicate significant difference of the means (P<0.01); n.s. indicates no significant difference of the means (P>0.05).

walls of the tank; adding food extracts. When stimulated by water jet streams, flicking rates increased to 0.15–0.9 flicks per second, although the animal generally remained in its position. Stimulating crayfish with jet streams of food extracts induced a higher frequency of flicking, at a rate of 0.5–1.5 flicks per second. In both groups, flicking rates during the three conditions (rest, non-odorant and odorant jet stream stimulations) were significantly different (Friedman test, $P<0.01$). Also in both groups, flicking rates during odorant jet stream stimulation were higher than the rates during non-odorant stream stimulation (Wilcoxon signed-rank test, $P<0.01$).

Ablation of the medial flagellum did not significantly reduce flicking rates whether the crayfish was at rest or whether it was stimulated with non-odorant or odorant jet streams (Mann–Whitney *U*-test, $P>0.05$). In all animals (intact, lateral-only or medial-only flagella), the introduction of food extracts consequently induced walking, antennal sweeping, raising of chelae, bottom searching of pereiopods and beating of mouthparts.

DISCUSSION

Mechanosensitivity of crayfish antennules

The antennules of *P. clarkii* have an abundance of mechanosensory setae. The procumbent plumose and medium simple setae comprise about 67% and 16% of the total number of setae, respectively. In crayfish, antennular setae with bimodal functions have yet to be identified. However, the procumbent plumose and medium simple setae are not known to be chemosensory. Procumbent setae, which respond to the bending of the segments that it spans, are not innervated (Tautz et al., 1981; Bender et al., 1984). Simple setae are possibly non-chemosensory because these setae did not absorb dye when tested for porosity (H.M.M., K.A., S. Uno, J. Koyama, T.M. and H. Y. Yan, unpublished). Aesthetascs comprise 9% of the total number of setae in crayfish antennules, which is lower than the 32% found in the antennules of the Caribbean spiny lobster *Panulirus argus* (Cate and Derby, 2001). When bimodal (hooded setae) and possible bimodal (long simple and medium setae) setae in lobster antennules are included, chemoreceptors would comprise 70% of the total number of setae in lobster antennules (Cate and Derby, 2001; Cate and Derby, 2002).

Medium simple setae were the most prominent on the stimulated area, i.e. the dorsal section on the distal half in both flagella. These setae are mechanosensory with bases that are supplied with dendrites and that are dually innervated (Chichibu et al., 1978b). The length of the shaft, the angle from the surface, and the socket of the shaft base determine the degree of deflection of the simple seta. The decrease in discharge rates when stimulated at high frequencies suggests that the shaft cannot follow the water motion induced by the moving sphere. As the surface of crayfish antennules probably does not generate a laminar flow, each receptor cell must be receiving different flow velocities. Chagnaud et al. (Chagnaud et al., 2008) showed that in fish lateral line, information about flow velocity and flow direction can only be provided when information received by an upstream afferent is coupled with information received by a downstream afferent. The distribution of the simple setae along the crayfish antennule, the frequency of their occurrence and their structure make simple setae well positioned to sample water movements, especially those coming from the anterior.

The results of this study demonstrate that the lateral and medial flagella of the crayfish antennules are both sensitive to hydrodynamic stimuli. The sensitivity of antennular mechanosensory setae is comparable to mechanoreceptors found on the chela of *Cherax destructor* (Tautz and Sandeman, 1980) and to the external joint receptors on the antennal flagella of *Astacus leptodactylus* (Tautz et al., 1981). However, these are less sensitive than mechanosensory setae that are associated with the reflex behavior of crayfish, such as those found on the tailfan (Wiese, 1976) and on another antennular seta, the standing plumose seta (Mellon and Christison-Lagay, 2008). The mechanosensory setae in crayfish have different peak responses. The low-pass and broad-band interneurons in the abdomen of *P. clarkii* were most responsive at 30 and 60 Hz, respectively (Plummer et al., 1986). On the first antenna of *A. leptodactylus*, the peak sensitivities of smooth conical and procumbent plumose setae were at 40 and 90 Hz, respectively (Tautz et al., 1981). By contrast, the peak response for the mechanosensory setae on the chelae of *C. destructor* was at 150 Hz (Tautz and Sandeman, 1980). Antennules of *P. clarkii* are most sensitive at 50 Hz, yet possess low-velocity thresholds between 30–130 Hz, thus neurons in the antennules can be classified as broad-band neurons (Plummer et al., 1986). In fish, velocity-sensitive superficial neuromasts have lowest thresholds in the frequency range of 20–60 Hz, whereas acceleration-sensitive canal neuromasts have minimal thresholds in the frequency range of 60–120 Hz (Kröther et al., 2002). It appears that the antennular mechanosensory properties of *P. clarkii* encompass the sensitive frequency ranges of the fish lateral line.

Relevance of mechanosensitivity in crayfish antennules

Crayfish antennules have hydrodynamic receptors with broadly tuned spectral sensitivity from 5–200 Hz. This sensitivity is in accordance with the low frequency nature of naturally occurring signals (Kalmijn, 1989; Bleckmann et al., 1991). Animal-borne water movements consist of short-lasting transients, long-lasting irregular oscillations, or a mixture of both (Bleckmann et al., 1991). Hydrodynamic flows caused by a dipole source can be perceived by fish (Montgomery et al., 1988; Coombs and Conley, 1997a), and they have the ability to localize this source (Coombs and Conley, 1997b; Coombs and Conley, 1997a). Thus the mechanosensory system of a fish, called the lateral line, has a direct relevance to its orientation to water currents (rheotaxis), as well as to prey detection and localization (Montgomery et al., 2002). Can crayfish use the same information to detect predator or prey? It has been shown that mechanoreceptors can perceive the swimming movement of fish, as demonstrated in the response of blindfolded *P. clarkii* (Breithaupt et al., 1995). Similarly, the Norway lobster *Nephrops norvegicus* responded to water particle displacement and showed a specific set of postural responses to sound frequencies of 20–180 Hz (Goodall et al., 1990). Owing to their position, it can be assumed that antennular mechanoreceptors provide information on the presence and movement of predators and prey that are present in front of the animal.

In addition to interspecific hydrodynamic signals, crayfish possess organs that produce flow fields and are employed in intraspecific communication. For example, the rhythmic beating of three pairs of flagella of the maxillipeds can generate a forward directed jet or can draw water towards the head region (Breithaupt, 2001). Another self-generated flow is produced when urine is carried by gill currents or by currents created by fanning the exopodites of the mouthparts (Breithaupt, 2001; Breithaupt and Eger, 2002). Previous studies demonstrated that urine release is olfaction related, i.e. urine signals are directed at opponents in fights (Breithaupt and Eger, 2002) and during courtship interactions (Berry and Breithaupt, 2010). The role of mechanoreceptors during such agonistic or courtship display was not elucidated but, in the snapping shrimp *Alpheus heterochaelis*, strength and fighting ability are assessed through the velocity of water jets received from opponents (Herberholz and Schmitz, 1998). Is it possible that antennular mechanoreceptors play a role in determining the outcome of agonistic and courtship interactions? In crayfish, urine signals during fight and courtship interactions are directed towards the antennules of the opponents (Breithaupt and Eger, 2002; Berry and Breithaupt, 2010). In such interactions, urine plays a role in status recognition (Zulandt Schneider et al., 2001; Horner et al., 2008). Because a crayfish can modulate the flow pattern it produces (Breithaupt, 2001), it can be presumed that antennular mechanoreceptors might use flow velocity and the frequency of urine release to assess the strength of the opponent or the viability of a potential partner. Further tests are needed to validate this hypothesis but the significance of the role of antennular mechanoreceptors during interactions between potential aggressors or mates cannot be ignored.

Antennular mechanosensitivity also plays a role in antennular activities such as flicking and depression. Flicking has consistently been induced by hydrodynamic stimuli, such as water jet streams, blowing and even tapping the tank walls. Mellon (Mellon, 1997) had the same observation and theorized that this is an energy-saving strategy, for it is more economical for the animal to scan the ambient waters with their olfactory receptors when they initially detect water movement around them. We support this theory further by our observations that stimulation with plain water jet streams evoked only flicking responses, whereas stimulation by food extract jet streams induced a longer flicking duration and induced food searching behaviors, such as walking, raising of chelae, and antennal, pereiopod and maxilliary movements.

Antennular activities in crayfish and difference in function between flagella

The low occurrence of chemosensory setae and the abundance of mechanosensory setae in *P. clarkii* antennules are surprising considering that antennules have primarily been reported to influence chemosensory-mediated behaviors in decapod crustaceans. The number of olfactory receptor neurons (ORN) in each aesthetasc is also low in crayfish. Crayfish and lobster aesthetascs have about 170 and 300 ORNs each, respectively (Mellon et al., 1989; Cate and Derby, 2001). It appears that the number of bimodal chemoreceptors in crayfish antennules is also lower than that found in lobster antennules. Daniel et al. (Daniel et al., 2008) showed that in spiny lobsters ablation of the entire antennular medial flagella attenuated the flicking response of lobster to food odors as a result of bimodal setae loss. By contrast, our results showed that ablation of the medial flagellum did not result in a significant reduction in flicking response. It appears that the loss of bimodal receptors, when the medial flagellum was ablated, was compensated by the mechanosensitivity of the lateral flagellum.

Our electrophysiological and behavioral data show that both the lateral and medial flagella are capable of detecting water motion. In addition, electrophysiological tests showed that both flagella respond to food odors (Mellon, 1997; Mellon and Humphrey, 2007). So, how do these flagella differ in function? In terms of structure, the lateral flagellum hosts the aesthetascs and their associated setae. In crayfish, the aesthetascs mediate intraspecific communication. *Procambarus clarkii* that lack aesthetascs fail to establish dominance hierarchies and continue to engage in fighting among other crayfish (Horner et al., 2008). Also, pheromone perception in *Orconectes propinquus* takes place in the lateral flagellum, possibly through the aesthetascs (Tierney et al., 1984). In terms of behavior, the lateral flagellum is the flicking organ. When at rest, crayfish antennules are seldom moved, which is in contrast to other crustaceans, e.g. hermit crabs, which continually flick their antennules (Snow, 1975). Results from our behavioral tests showed that although non-odorant jet streams also evoked flicking, the frequency of flicking increased when odorant streams were introduced. This suggests that the crayfish was actively probing the ambient waters by flicking the lateral flagella for possible localization of chemical cues. In spiny lobsters, the ablation of the aesthetascs resulted in the complete attenuation of flick rates (Daniel et al., 2008). Flicking the aesthetasc-bearing lateral branch of the antennule is a behavior exhibited by decapod crustaceans, and is associated with the detection of chemical cues in the ambient water environment. Downward flicking splays out the aesthetascs, thereby flushing the entrapped water and allowing the capture of novel odorant-bearing water samples during the slower return stroke (Snow, 1973; Schmitt and Ache, 1979; Koehl et al., 2001). Because flicking enhances olfaction by decreasing the thickness of the boundary layer to allow molecules to reach receptors, then minimum velocities are necessary to facilitate penetration of odor molecules through the surrounding fluid (Koehl, 2006). Mellon and Humprey (Mellon and Humprey, 2007) and Humphrey and Mellon (Humphrey and Mellon, 2007) showed that simple setae on the dorsal and ventral aspects of the lateral flagella experience asymmetries in drag and torque during the downward and upward flick cycles. These simple setae on the lateral flagellum should play a very important role by providing feedback on the velocity of the downstroke during the flick.

The medial flagella in *P. clarkii* antennules are not involved in flicking. Still, the medial flagellum is also capable of detecting food odors, probably through non-aesthetasc setae that might have bimodal functions (Cate and Derby, 2001; Cate and Derby, 2002; Schmidt and Derby, 2005). However, its extra sensitivity to hydrodynamic cues suggests that this organ is the best candidate for the detection of fluid motion. There are several arguments as to why its role as a hydrodynamic detector is just as important and should be underscored. Morphologically, the medial flagellum is just as prominent as the lateral flagellum, even slightly longer, which hints that its role in the sensory process is just as important. In addition, the absence of chemoreceptive aesthetascs and the presence of a fairly large number of mechanosensory setae in the medial flagellum also emphasize this role. Physiologically, its extra sensitivity to hydrodynamic stimuli should be recognized. The complementing roles of the biramous antennules are illustrated in the depression of the antennules during non-odorant and odorant stimulation. When presented with stimuli, especially food extracts, the crayfish started to beat its maxillipeds. Instead of simply flicking to search the stimulus source, the crayfish spent time by lowering a pair or all of the antennules towards the maxillipeds. When depressed, the ventral surface of the distal halves of the antennules, i.e. the aesthetascs in the lateral flagellum and the simple setae on the medial flagellum, is exposed near the maxillipeds. Beating the maxillipeds allows crayfish to regulate flow patterns (Breithaupt, 2001; Denissenko et al., 2007) and to draw water towards the aesthetascs and simple setae. In this behavior, it appears that aesthetascs act as chemosensors for relevant odor molecules (Breithaupt, 2001; Denissenko et al., 2007). We expect that the simple setae that are present all over the distal half of the medial flagellum could monitor the direction and velocity of the flow. The same mechanism occurs in fish: localization of food sources involves the use of nares (olfaction) and the lateral line (mechanoreception), although, if the gustatory system is controlling the behavior, chemical and mechanical sensors are co-localized to allow orientation to the source.

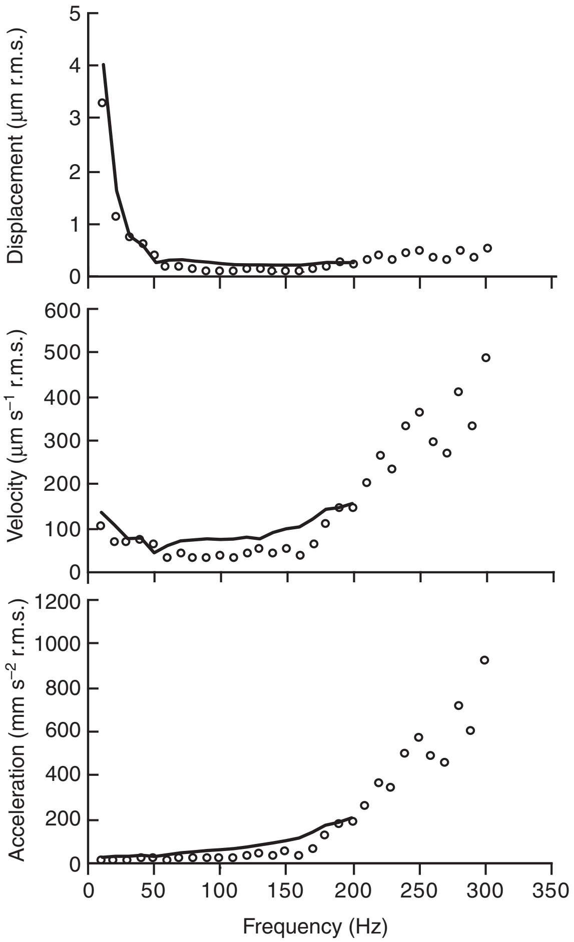

Fig. 6. Comparison of the threshold of the crayfish medial antennular flagellum and of the fish lateral line [after Watanabe and Anraku (Watanabe and Anraku, 2007)] to displacement, velocity and acceleration of water particle motion. Circles and solid lines indicate fish lateral line and crayfish antennular medial flagellum thresholds, respectively.

Mellon (Mellon, 2005) reported that different afferent pathways are involved in antennular responses to hydrodynamic and odorant stimuli, and that the multimodal integration of chemical and mechanical information occurs at the level of first-order sensory interneurons in the crayfish brain. This multimodality of the biramous antennules should be useful to crayfish because, compared with a single sense, the synchronous use of different faculties provides the animal with more reliable information. The integration of hydrodynamic, chemosensory, tactile and visual information becomes a powerful tool, especially in prey capture. It becomes more beneficial to crayfish because this animal lives in dark areas where visual information might not be available and in environments in which stagnant flow conditions curtail odor transport.

Crayfish antennules as part of a fish lateral line analog?

The highly sensitive nature of crayfish antennules to hydrodynamic stimuli reminded us of the sensitivity of the fish lateral line that researchers in our laboratory had observed using the same method of stimulation. Fig. 6 shows the thresholds of the carp lateral line to displacement, velocity and acceleration of water particle motion, as reported by Watanabe and Anraku (Watanabe and Anraku, 2007), against those of the crayfish medial antennular flagellum. The similarity of response is striking, which led us to ask: is the crayfish antennule an analog, or does it at least form part of an analog, of the fish anterior lateral line? The fish anterior lateral line is the mechanosensory system present in the head of the fish, which consists of numerous receptors located either on the body surface (superficial neuromasts) or in the subepidermal canals (canal neuromasts) (e.g. Coombs et al., 1988; Bleckmann, 1993). It appears that aside from the similarity in threshold, the basic mechanism of cell response to fluid motion might also be similar. Both systems employ an erect structure, a shaft (in crayfish antennules) and a kinocilium or cupula (in fish lateral line), which, when displaced by fluid motion, has its cell membrane potentials modulated. Of course, the detailed and minute mechanisms might not be the same, and it is yet unclear how the mechanosensory information received from all over the body of the crayfish (e.g. the thorax, chelae, pereiopods, tail, antennae) are integrated, but other researchers have long raised the probability of a lateral line analog in crustaceans (e.g. Wiese, 1976; Guenther and Atema, 1998; Popper et al., 2001). Such an analog has also been reported in cephalopods (Budelmann and Bleckmann, 1988), and a similar convergence in evolution between a crustacean and a vertebrate sensory system might be possible. Further morphological, physiological and behavioral data on crustaceans will contribute to our understanding of fish and crustacean mechanosensory systems.

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