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RESEARCH ARTICLE

Identified antennular near-field receptors trigger reflex flicking in the crayfish

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SUMMARY

Near-field disturbances in the water column are known to trigger reflex antennular flicking in the crayfish *Procambarus clarkii*. We have identified the hydrodynamic sensors on the lateral antennular flagellum that constitute an afferent limb of this reflex and have measured the relative directionally dependent thresholds of the sensory neurons associated with these structures to hydrodynamic stimulation. Twenty-five individual standing feathered sensilla, comprising a sparse, linearly arrayed population of near-field sensors along the lateral and medial antennular flagella, were exposed to standardized pulsatile stimuli at 20 deg intervals along a 320 deg circular track. The results indicate that the sensilla are most sensitive to such stimulation in the plane of the flagellar axis. Identification and mechanical stimulation of single feathered sensilla in some preparations consistently evoked a flick reflex at maximal response latency, indicating that these sensors constitute at least one afferent limb for the reflex behavior. Experiments in which response latencies were measured following mechanical stimulation of truncated flagella, and were compared with the latencies in respective intact flagella, suggest that summation of inputs from the feathered sensillar pathways generates reflex flicking at minimal latencies. We discuss the possible central mechanisms that may underlie detection of critically important signals from this population of highly sensitive, inherently noisy sensors.

Key words: antennule, behavior, Crustacea.

INTRODUCTION

Almost without exception, decapod and stomatopod crustaceans engage in antennular flicking, a behavior that theoretically enhances odorant detection through increasing the leakiness of the array of antennular olfactory (aesthetasc) sensilla to odorant-bearing fluid filaments (e.g. Snow, 1975a; Snow, 1975b; Schmitt and Ache, 1979; Mellon, 1997; Koehl et al., 2001; Mead and Koehl, 2000; Stensmyr et al., 2005; Mellon and Reidenbach, 2011). The very small size of aesthetasc sensilla (10 μm in diameter at the base by 100 μm long in crayfish) dictates that their Reynolds numbers – a measure of the ratio of inertial to viscous fluid forces – will also be small. Fluid flow around these structures is therefore laminar, and the fluid boundary layer accompanying each of the aesthetasc sensilla will accordingly be relatively thick, except when the lateral flagella are flicked through the fluid medium at a high enough velocity to dynamically shed some or most of it. By reducing the thickness of the boundary layer around individual aesthetascs, flicking promotes access of odorant-laden fluid to the cuticular surface of the sensilla (Cheer and Koehl, 1987; Goldman and Koehl, 2001; Koehl et al., 2001; Reidenbach et al., 2008; Mellon and Reidenbach, 2011). This behavior thereby improves the diffusional exposure of olfactory receptor neuron dendrites within the aesthetascs to odorants embedded in the fluid column. Previous work indicated that hydrodynamic stimulation of the lateral antennular flagellum evoked a flicking reflex in the crayfish *Procambarus clarkii* (Mellon, 1997). A subsequent study (Mellon and Christison-Lagay, 2008) then identified a specific population of near-field receptors, the standing feathered sensilla, linearly arrayed along the antennular flagella. We wished to confirm that weak hydrodynamic stimuli are able to excite the feathered sensilla, and to determine whether this specific population of sensors might trigger the flicking reflex previously found to be evoked by hydrodynamic stimuli.

In crayfishes, aesthetasc sensilla are arrayed uniformly, but comparatively sparsely, along the ventral surface of the distal half of the lateral antennular flagellum (Tierney et al., 1986; Mellon et al., 1989). Individuals of *P. clarkii* flick their lateral antennular flagella ventrally from their normally near-vertical posture in response to mechanical stimulation of the head region or its appendages including, as stated above, hydrodynamic stimulation of the lateral antennular flagellum (Mellon, 1997). Turbulent fluid shear sensed by the antennular sensors is an effective flick-inducing stimulus, presumably as a response to the potential for imbedded odorants. Because of the natural curvature of the crayfish lateral flagellum, its normal posture and its distal flexibility, fluid dynamic models suggest that there will be a net movement of water through the array of aesthetascs during the downward flick from proximal to distal, that is, from the flagellar base toward its tip (Humphrey and Mellon, 2007), and recordings from crayfish brain neurons that respond to antennular hydrodynamic receptors as well as chemical stimulation of the aesthetascs indicate that a proximal-to-distal flow is the most effective stimulus (Mellon and Humphrey, 2007).

As with other crustaceans, flicking in *P. clarkii* is a phasic behavior; it is generated by action potential bursts in a single motor neuron that supplies a substantial portion of the lateral (external) flagellar depressor muscle (EDM), resulting in excitatory junctional potentials (EJPs) that, at burst frequencies, facilitate rapidly (Mellon, 1997). The return stroke, however, is apparently driven through energy stored during the initial phase of the flick in an elastic ligament at the base of the lateral flagellum. There is no flagellar extensor muscle. Fig. 1 shows four video frames of a sequence filmed during a spontaneous antennular flick in *P. clarkii*, indicating the

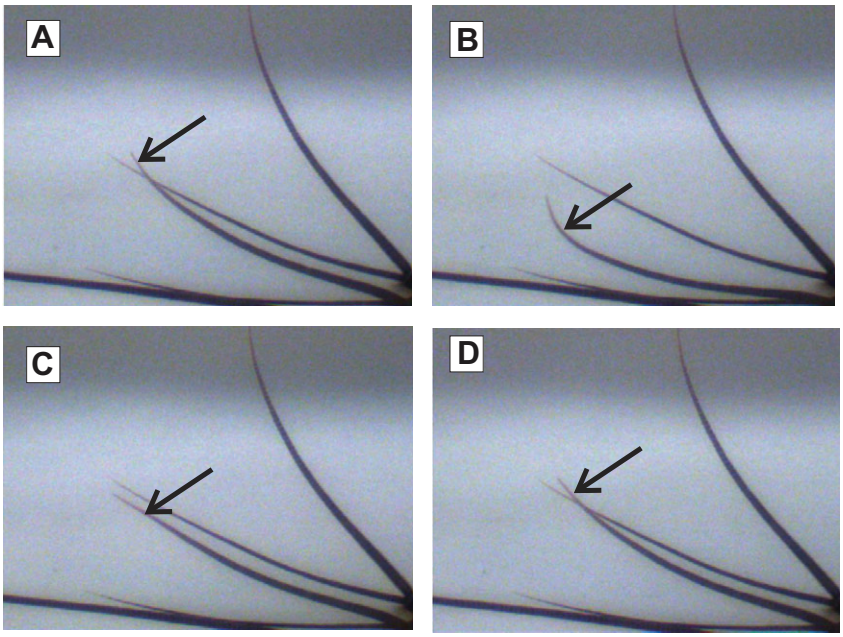

Fig. 1. Single frames from a crayfish (*Procambarus clarkii*) antennule flick sequence. (A) Left lateral flagellum (arrow) just prior to beginning the flick. In B, the flagellum is at its ventral-most position, whereas C and D illustrate the flagellum approximately halfway through the return stroke and immediately after regaining its resting posture, respectively. The left medial flagellum is just visible at the bottom of each frame, adjacent to the left second antenna. Modified from Mellon and Reidenbach (Mellon and Reidenbach, 2011).

flagellar posture at the start, at the end and in motion during a single flick. Especially obvious is the flexibility of the flagellum during the initial phase and, in consequence, its passive bending response to viscous drag exerted upon it during the downward motion. Identical flicks can be evoked through mechanical stimulation, as stated above.

Despite numerous studies on the fluid mechanics of flicking behavior, questions remain concerning its generation, strength, frequency and response latency. The sensors comprising the afferent limb of the crayfish flagellar flick reflex in response to antennular stimulation have not been previously identified behaviorally or through examination of their central synaptic connections with EDM motor neurons. In this paper we present electrophysiological and behavioral evidence that standing feathered sensilla on the lateral antennular flagella, sensors that are very sensitive to directly imposed mechanical disturbances (Mellon and Christison-Lagay, 2008), respond readily to water particle motions, and constitute a specific afferent pathway that triggers antennular flicking. In addition, we present circumstantial evidence that spatial summation of inputs from the array of feathered sensilla decreases the latency of the flick response.

MATERIALS AND METHODS

Animals used for physiological study were large adult southern swamp crayfish, *Procambarus clarkii* (Girard 1852), obtained from a commercial source in Louisiana (Atchafalaya Biological Supply, Raceland, LA, USA). They were kept in a circulating freshwater culture system (Marine Biotech, Inc., Beverly, MA, USA) maintained on a 12 h:12 h light:dark schedule at 20°C and fed twice weekly on frog brittle (Nasco, Fort Atkinson, WI, USA) until used.

Scanning electron micrographs were obtained from excised lateral antennular flagella of small (5 cm) 24 h postmolt or intermolt animals. Postmolt animals had a cleaner cuticle, i.e. they were not contaminated with debris or bacteria. Flagella were surgically removed from the animals, fixed overnight at 4°C in 2% paraformaldehyde–2% glutaraldehyde in 0.1 mol l^{-1} sodium phosphate buffer (pH 7.4). Fixed flagella were washed 3× in 0.1 mol l^{-1} phosphate buffer, dehydrated in an ethanol series and subjected to critical-point drying before being gold coated and examined on a JEOL 6400 scanning electron microscope (JEOL Ltd, Tokyo, Japan).

Fig. 2 illustrates the recording arrangement for determining feathered sensilla sensitivity to hydrodynamic stimulation. A square Lucite® recording chamber (10×10 cm) filled with crayfish saline was used as the stimulating arena; a suction-capable electrode holder was fitted through one wall of the chamber to accommodate a short length of 1.0 mm diameter glass capillary tubing, the end of which was near the center of a circular array of black marks on the chamber floor spaced at 20 deg intervals from each other. The capillary tubing was coupled to the base of an excised crayfish lateral antennular flagellum by means of a short length of appropriately sized latex tubing, and the electrode holder was then connected to a low-level AC amplifier (Grass model P511, AstroMed Inc., West Warwick, RI, USA), an oscilloscope, a digitizer and audio equipment. Spiking activity from neurons associated with standing feathered sensilla, based on amplitude and waveform following stimulation with a small probe (Mellon and Christison-Lagay, 2008; Mellon, 2010), was used to identify a specific sensillum under ×100 magnification. The flagellum was then truncated one annulus distal to the sensillum, and saline was gently drawn through the flagellar blood sinus to flush it. Hydrodynamic stimuli were supplied by a polystyrene sphere 9.5 mm in diameter and immersed in the saline bath at a level 1 cm above that of the sensillum, and sequentially aligned with the stimulus positions arrayed circularly around the sensillum (see Fig. 2A). The sphere was attached to a plastic rod that was driven vertically by a small audio speaker (Fig. 2B). Electrical pulses 5 ms in duration were delivered every 10 s to the speaker, for 10 trials at each 20 deg mark around the sensillum, except at the 180 deg point; stimulus intensity (voltage output to the speaker) was adjusted to just-suprathreshold amplitude. Each complete series of trials was then repeated, and the results at each stimulation position from the two trials were averaged. Data from each sensillum were normalized to maximum stimulus threshold, and the mean normalized values, ±1 s.e.m., from all preparations, were plotted on polar coordinates. Stimulus voltage intensity was converted to probe movement using the empirical relationship we obtained, shown in Fig. 3.

Perfused isolated head preparations were employed to measure response parameters of the flick reflex; antennular flicking is

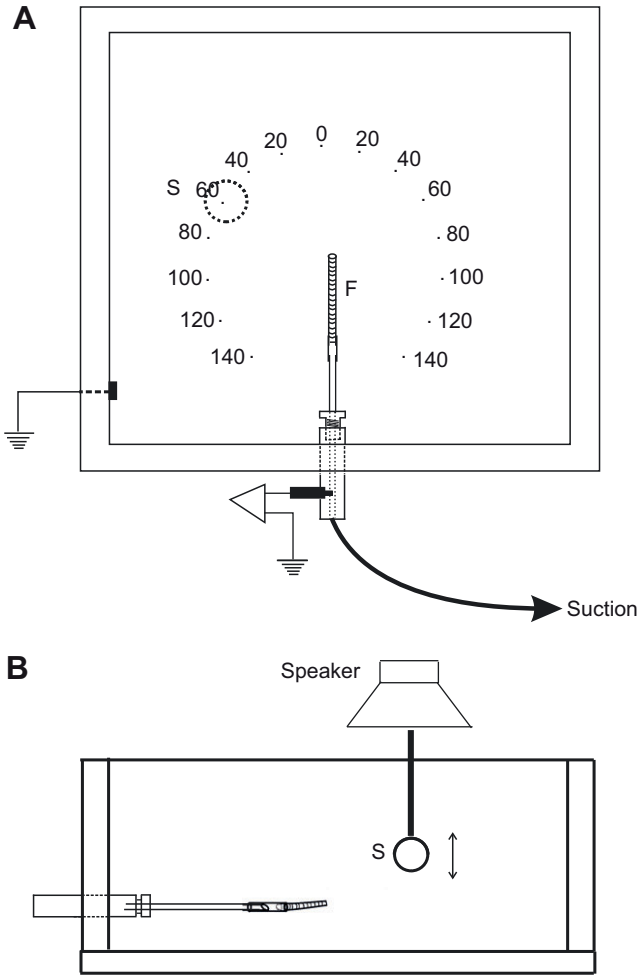

Fig. 2. Experimental arrangement used to measure spiking thresholds from identified standing feathered sensilla in the crayfish *P. clarkii*. (A) Overhead view of the recording chamber, indicating the truncated lateral flagellum (F), attached to a suction electrode, and the stimulus positions surrounding a sensillum at the distal end of the truncated flagellum. The dotted circle on the 60 deg left position (S) indicates the relative size of the stimulus sphere compared with its distance from the sensillum within the fluid-filled chamber. (B) Side view of the sphere and truncated flagellum, showing their relative vertical positions.

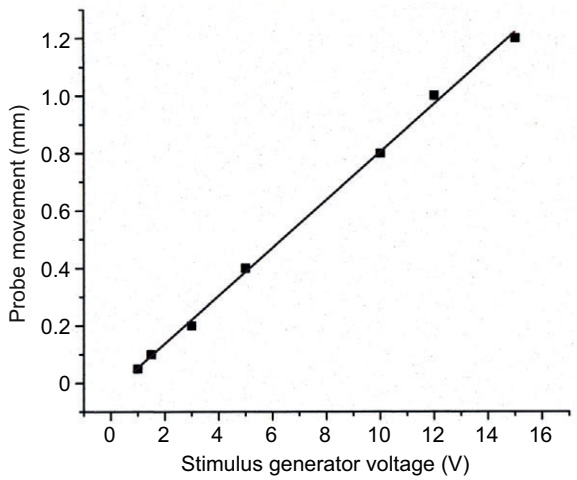

Fig. 3. Experimentally derived plot of probe movement *versus* stimulus generator voltage.

quickly abolished in the absence of a functioning brain circulatory system. Crayfish were chilled on ice for 20–30 min to anesthetize them. They were then decapitated by cutting through the cephalothorax at the cervical groove. The rostrum was removed and the stomach and green glands were dissected away. After mounting the head 45 deg to the vertical and with the ventral aspect of the antennular flagella up on a SYLGARD® shelf in a recording chamber, the median artery was cannulated at the *cor frontale* with a tapered, fire-polished glass capillary tube, and the brain was perfused with chilled (17°C), oxygenated crayfish saline (composition, in mmol l^{-1}: 205 NaCl; 5.4 KCl; 13.6 $CaCl_2 \cdot 2H_2O$, 2.7 $MgCl_2 \cdot 7H_2O$, and 2.4 $NaHCO_3$; pH adjusted to 7.4 with HCl). Next, a ventral window was cut in the third basal segment of the left antennule to expose the EDM. An extracellular suction electrode, made from a fire-polished capillary tube and connected to an AC recording system, as above, was gently lowered onto the surface of EDM and moved around until suitably sized motor nerve plus EJP bursts were observed in response to mechanical stimulation of the ipsilateral lateral flagellum (mechanical stimuli applied to the medial flagellum were not effective in generating the flick reflex), at which time gentle suction was applied to the electrode to stabilize it. A ×100 dissecting microscope was then used to locate and visually control the position of a small stimulation probe with reference to individual standing feathered sensilla along the ventrolateral aspect of the lateral flagellum. Probes consisted of minuten nadeln bent at a 90 deg angle at their tip and coupled to a miniature audio speaker. The speaker was driven through a stimulus isolation unit by an electrical stimulus generator (Grass model S48, Astro-Med Inc., West Warwick, RI, USA). In some experiments the probe was used to mechanically stimulate the entire lateral flagellum. Responses to 10 individual stimulus trials, each separated by 30 s, were stored in computer files for later measurement and analysis.

Comparisons of evoked axonal response latencies, between mechanical stimuli delivered to single feathered sensilla and the flagellum itself, were obtained from non-perfused isolated head preparations by recording from the lateral fascicle of the antennular nerve at its entrance to the brain. An isolated head was mounted ventral side up in a suitable recording chamber that was continuously flushed with crayfish saline at 17°C, and the antennular nerve was exposed at its entrance to the brain. The lateral branch of the nerve was identified, and a suction electrode was used to record spiking activity from feathered sensilla. Visually identified feathered sensilla were stimulated with a small speaker-driven probe, and their specific spike waveforms were identified and recorded in response to 10 individual suprathreshold 2 ms mechanical stimuli, each separated in time by 5 s. The probe was then used to stimulate the flagellum at the same position with the same parameters, and the responses to 10 identical stimuli were recorded and stored. Comparisons of initial response latencies to the two modes of stimulation were tabulated as means ± s.e.m. and analyzed using *t*-tests.

Perfused isolated head preparations were used to compare response latencies following stimulation applied at the basal segment of an intact lateral flagellum with those of the same flagellum following sequential removal of two approximately isometric distal segments. EJP burst latencies were recorded from the dorsal surface of the EDM *via* an extracellular suction electrode. EJP burst latencies were obtained from the intact flagellum, following which

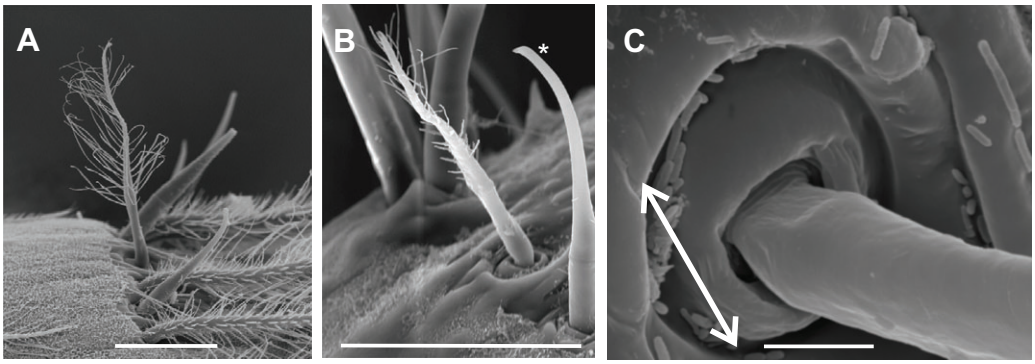

Fig. 4. (A,B) Typical standing feathered sensilla on the lateral flagellum of *P. clarkii* showing the characteristic 'dog leg' that occurs two-thirds of the way distally along the shaft; on the sensillum shown in B, many of the normal filaments, evident in A, have been worn down and better reveal this feature. The structure shown in B marked with an asterisk is a beaked sensillum (Mellon and Humphrey, 2007), a type variously identified in *P. clarkii* as a 'simple seta' (Monteclaro et al., 2010) and as 'guard hairs' or 'companion hairs' in the crayfish *Cherax destructor* (Sandeman and Luff, 1974). (C) Details of the socket of a feathered sensillum at high magnification. The double-headed arrow indicates the preferred directional sensitivities of the neurons associated with the sensillum, at right angles to the hinge-like elongation of the sensillar base. The rod-like objects are bacilli, which are sometimes associated with the cuticle of intermolt animals. Scale bars, (A,B) 100 μm; (C) 5 μm. Panel A is modified from Mellon and Christison-Lagay (Mellon and Christison-Lagay, 2008).

approximately 33% of the distal portion of the flagellum was surgically removed. After responses to ten stimuli were obtained and stored, an additional 33% of the flagellum was removed, and response latencies with the remaining proximal 33% of the flagellum were tested. Linear regression was used to analyze the results.

RESULTS

Sensitivity of sensilla to water particle displacement

Previous results (Mellon, 1997) indicated that lateral flagellum flicking is reflexively triggered by pulsed hydrodynamic stimulation, following the activation of sensory neuron axons in the flagellum, which exhibited very large spike amplitudes. We later inferred that these axons innervate the standing feathered sensilla examined recently in *P. clarkii* (Mellon and Christison-Lagay, 2008), which are sparsely arrayed, bidirectional near-field receptors found along the ventral edges of both the lateral and medial flagella of the antennules. Typical features of standing feathered sensilla from the lateral flagellum are shown in the scanning electron micrographs of Fig. 4. The shaft of the sensilla has a discernible 'dog-leg' feature approximately two-thirds of the way along the shaft, and it is characterized by a planar array of flat, ribbon-like filaments, a property similar to that previously found on tailfan near-field sensilla in *P. clarkii* (Wiese, 1978; Douglass and Wilkens, 1998).

To confirm that the feathered sensilla do in fact respond to near-field stimulation, we examined spike thresholds of identified individuals on isolated, truncated lateral flagella to single, pulsatile hydrodynamic stimuli moved around the sensilla through an arc of approximately 320 deg. The amplitude of 5 ms mechanical pulses delivered beneath the surface of a saline bath was varied until they

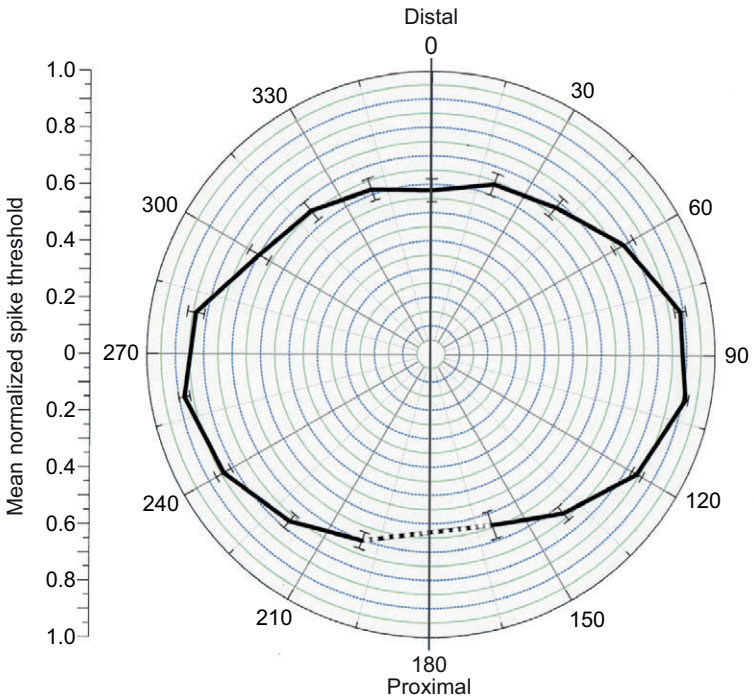

Fig. 5. Polar plot of normalized sensillar spiking thresholds in *P. clarkii* as a function of the relative position of a 5 ms near-field stimulus. Data are means ± 1 s.e.m. of 25 sensilla. The 0 deg position was directly ahead of (distal to) the sensillum under observation. The stimulus probe was moved sequentially in 20 deg increments to 17 different positions clockwise and counterclockwise around the sensillum. (The dotted line indicates the sector where threshold measurements could not be made.) The results indicate a slightly but significantly greater sensitivity (lower thresholds) of the feathered sensilla to near-field stimulation parallel to the flagellar axis ($P<0.001$).

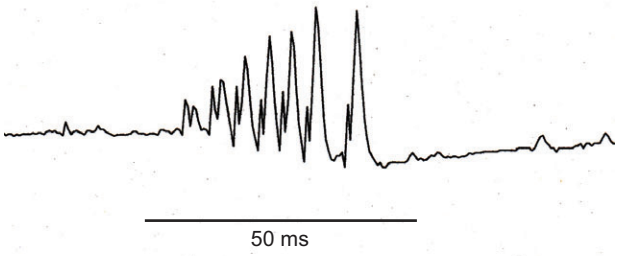

Fig. 6. Extracellular records of an excitatory junctional potential (EJP) burst in (external) flagellar depressor muscle (EDM) of *P. clarkii*, evoked by mechanical stimulation of a single feathered sensillum. Sharp deflections immediately prior to each EJP are motor neuron spikes, which, unlike the rapidly facilitating (ca. 6× in this example) junction potentials, have a fixed amplitude. Most EDM bursts consist of six to eight individual EJPs.

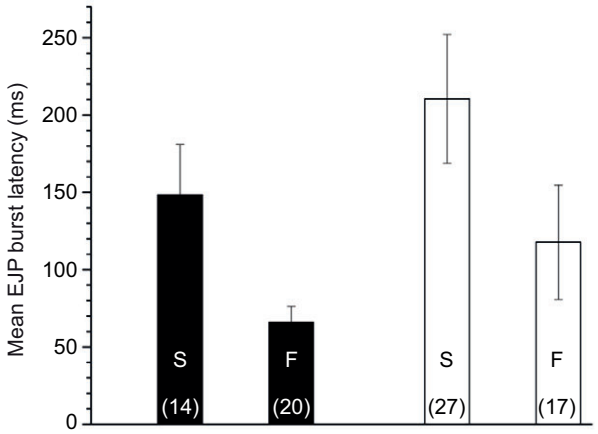

Fig. 7. Mean (±s.d.) EJP burst latencies in the EDM in response to stimulation of a single feathered sensillum (S) or the flagellum (F) in two (open and filled bars, respectively) isolated perfused crayfish (*P. clarkii*) head preparations. Numbers within parentheses in the bars indicate the number of observations from which the mean values were computed.

produced consistent, barely suprathreshold spiking in one or both sensory axons associated with the identified sensillum. We obtained reliable data from 25 individual sensilla, whose mean ± s.e.m. normalized threshold data are shown in the polar plot of Fig. 5. The data indicate that the sensilla are maximally sensitive, i.e. have the lowest thresholds, to impulsive stimuli originating close to the axial plane of the flagellum, as suggested by previous observations of spiking responsiveness using direct sinusoidal mechanical stimulation of the sensillar shaft (Mellon and Christison-Lagay, 2008). As we previously observed, however, the differences in directional sensitivities were not especially acute, except for a definite drop close to 180 deg opposite the preferred direction, and they were even less so using near-field stimulation techniques, in which hydrodynamic disturbances close to the antennules originating from any direction are easily sensed. However, an ANOVA analysis of the data indicates highly significant differences in response threshold at different angles with reference to the sensilla ($P<<0.001$). A *post hoc* analysis (Tukey's honestly significant difference) of the response thresholds at 0 and 80 or 100 deg and at 0 and 260 or 280 deg indicated that the differences were significant ($P<0.001$). We were not able to directly measure or find a suitable numerical model solution for water particle movements transmitted to the sensilla following their generation by the stimulus sphere. In terms of thresholds to known movements of the sphere itself, individual sensilla from different preparations were quite variable, ranging over two orders of magnitude: threshold movements varied from a high value of 0.73 mm movement at a distance from the sensillum of just 4 mm to a low value of 0.05 mm movement at a distance of 2.3 cm.

Response latencies following stimulation of individual feathered sensilla and the flagellar shaft

To determine whether standing feathered sensilla constitute an afferent limb of the hydrodynamically generated flicking reflex, we stimulated individual sensilla directly with a speaker-driven probe to evoke reflex flicking in perfused isolated crayfish head preparations. Fig. 6 illustrates the characteristic motor neuron spike and EJP bursts obtained with extracellular recording techniques following abrupt mechanical stimulation of a single feathered sensillum. The pronounced synaptic facilitation is also a characteristic property of these neuromuscular junctions (Mellon, 1997). In the preparation from which the response shown in Fig. 6 was obtained, which is typical, EJP amplitude increases of up to eightfold occurred from the first motor neuron spike to the final spike of the burst.

In most preparations, we found that stimulation of individual sensilla was without effect as a trigger for EDM bursts, even though we know from previous studies (Mellon and Christison-Lagay, 2008; Mellon, 2010) that the identical method of mechanical stimulation is very effective in generating spikes in feathered sensillar neurons. The procedure for arterial cannula insertion in the inverted head position is difficult and time consuming. Most of the head preparations attempted in this configuration failed to generate reflex flicking at all, presumably because of a lack of brain circulation during the inordinately long cannulation procedure. Furthermore, among those preparations that did remain viable, few exhibited reflex flicking to stimulation of a single individual feathered sensillum. We did, however, obtain observations in two favorable preparations in which a mechanical pulse delivered to a single identified sensillum was sufficient to evoke consistent one-for-one reflex bursts of activity in EDM. These findings, even though based upon only two individuals, confirm that standing feathered sensilla represent at least one population of afferents that can trigger reflex flicking. The reflex response latencies observed following stimulation of single sensilla were, however, nearly twice as long as those following mechanical stimulation of the flagellum as a whole. Latency data from the two successful examples are shown in Fig. 7. In each case, the sensillum was identified visually with a ×100 dissecting microscope and it was confirmed that specific physical contact with the probe evoked EJP bursting. Single 2 ms electric pulses to the speaker at just-suprathreshold intensities generated an EJP burst in EDM at maximum latency, in the two cases shown at approximately 150 or 210 ms, respectively, following the mechanical pulse. Extreme care was taken that the stimulus probe was not acting on the flagellum itself or on any other neighboring sensillum type. Displacing the probe laterally, so that its movement during a pulse was not in line with the feathered sensillum, or increasing its distance from the sensillum failed to evoke a response from EDM, suggesting that only the feathered sensillum under observation was providing the input for the response. In both preparations shown in Fig. 7, when the stimulus was directly applied to the flagellum at a point near the sensillum, the response latency was reduced dramatically, presumably as a result of effects of

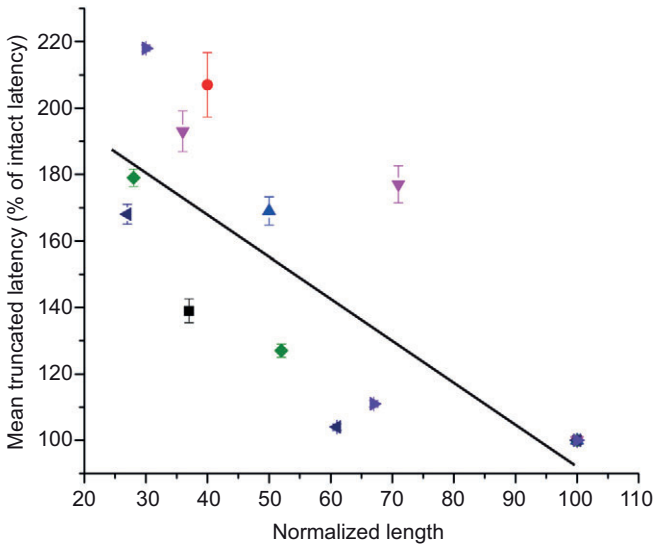

Fig. 8. Mean (±s.e.m.) EDM burst response latencies of truncated flagella from seven different preparations of *P. clarkii*, each indicated by different colored symbols. In three preparations, the flagellum was truncated roughly halfway along its length. In the remaining four preparations it was sequentially truncated twice. The best fit for the data is given by the equation $y=-1.2x+216$ ($r^2=0.37$, $P=0.05$). See Results for further explanation.

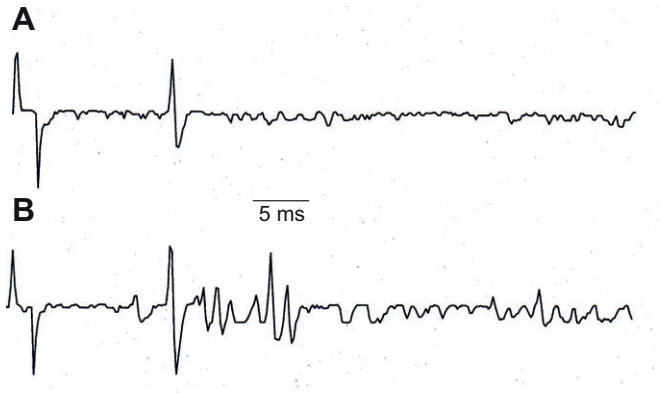

Fig. 9. Extracellular recordings of axonal spiking recorded from the antennular nerve of *P. clarkii* near its entrance to the brain. (A) The response to brief (2 ms) mechanical stimulation of a standing feathered sensillum. (B) The stimulus probe was placed against the flagellum adjacent to the sensillum and exposed to the same stimulus, generating activity in several sensory axons. Bipolar stimulus artifacts occur at the start of each sweep.

simultaneous, indirect stimulation of several or all of the standing feathered sensilla along the flagellum.

Input summation dependence of the response latency is further suggested by truncation experiments. We measured reflex latencies following mechanical stimulation applied to the basal annulus of an intact flagellum, and we subsequently compared them with reflex latencies obtained from the same stimuli after removing the distal half of the flagellum. In other experiments we compared the intact response latencies with those following removal of first the distal one-third of the flagellum and then the next proximal one-third. Results from both experimental procedures, involving seven different preparations, are shown in Fig. 8. Truncated response latencies, as a percent of the latency of the intact flagellum, are plotted against the normalized flagellar length. The graph indicates a weak inverse correlation between latency and length. Although, with this mode of stimulation, it is impossible to rule out possible influences in reflex generation from joint receptors in the three basal segments of the antennules, the increased response latency following truncation suggests that flagellar receptors are primarily implicated. Furthermore, removing sections of the flagellum reduces not only its mass but also its viscous drag, while the stimulus strength and duration remained constant. If phasic antennular joint receptors were involved in generating the reflex bursts, the truncation procedure should have improved the stimulus parameters and reduced the response latency; instead, the opposite was observed.

Additionally, just to confirm that direct mechanical stimulation of the entire flagellum does indeed recruit activity in multiple standing feathered sensilla, we recorded spiking activity from the lateral fascicle of the antennular nerve near its entry to the brain in four separate preparations in response to 2 ms mechanical pulses applied to individual identified feathered sensilla, and we compared them with spike responses, recorded immediately afterward, generated by identical stimuli delivered to the flagellum at that location. Results from a typical preparation are illustrated in Fig. 9. Although the individually stimulated sensilla responded with easily and uniquely identifiable spike waveforms measured at the brain (Fig. 9A), confirmed in some initial recordings by damaging the sensillum and thereby removing that spike waveform, stimulating the flagellum itself generated multiple overlapping waveforms (Fig. 9B), the initial spike of which had a mean latency that was usually (but not always) only slightly shorter than that following single sensillar stimulation (Table 1). Of course, stimulation of the entire flagellum would, on average, have activated some feathered sensillar neurons that may have had slightly shorter conduction times at the brain, accounting for the shorter latencies recorded under those conditions. The data do not, however, support the contention that the much shorter latencies of the muscle response following flagellar stimulation are due to some flagellar sensory pathway that has only half of the response latency than that of the standing feathered sensilla.

These observations not only point to the standing feathered sensilla as a sufficient afferent pathway for reflex flicking, but also indicate that spiking activity in only two, and possibly just one, sensory neuron(s) can be sufficiently effective in some preparations to evoke a flick. These findings also show that, following direct mechanical stimulation of the flagellum, activity from several sensilla is recruited at a latency at the brain little different from that following stimulation of individual feathered sensilla.

DISCUSSION

The results presented in this paper indicate that standing feathered sensilla along the lateral antennular flagellum of *P. clarkii* are highly sensitive hydrodynamic receptors that can trigger antennular flicking, and comprise a concluding chapter to a group of previous studies in which hydrodynamic antennular stimulation was shown to evoke reflex flicking in *P. clarkii* (Mellon, 1997), followed by identification of a class of near-field receptors on the antennular flagella of this species (Mellon, 2010; Mellon and Christison-Lagay, 2008). The results, furthermore, substantiate and extend the previous observations with these sensilla, primarily obtained with direct mechanical stimulation techniques (Mellon and Christison-Lagay, 2008). The bidirectional sensitivities of antennular standing feathered sensilla to water particle movements are, moreover, similar to the response profiles of crayfish tailfan near-field receptors examined by Douglass and Wilkins (Douglass and Wilkins, 1998).

Table 1. Comparisons of spiking response latencies (means ±1 s.e.m., *N*=10 trials per latency measurement), measured at the brain, from stimulating individual standing feathered sensilla and the flagellum

	Prep ID								
	6/20	6/22 A	6/22 B	6/22 C	6/24 A	6/24 B	6/24 C	6/27 A	6/27 B
Sensillum latency (ms)	8.4±0.08	6.57±0.07	6.66±0.14	8.06±0.07	11.34±0.05	12.77±0.09	12.37±0.12	6.82±0.05	7.5±0.08
Flagellum latency (ms)	6.2±0.04	6.6±0.04	6.51±0.04	7.74±0.08	9.02±0.28	11.36±0.21	10.53±0.18	6.32±0.07	7.18±0.14
Difference	+2.2	–0.03	+0.05	+0.32	+2.32	+1.41	+1.84	+0.5	+0.32

Small differences in arrival times are seen, with the multiple-spike latencies following direct flagellar stimulation usually being somewhat shorter.

Although we were not able to directly measure the spiking thresholds of individual feathered sensilla in terms of water particle movements, their relative directional thresholds indicate a preference for near-field stimuli propagating in line with the flagellar axis. Turbulent eddies within the water column, including those with dissolved odorants, will create fluid shear that would be expected to excite standing feathered sensilla and thereby trigger antennular flicks, ostensibly improving odorant capture. Our observations provide evidence that the standing feathered sensilla constitute at least one afferent pathway for reflexive antennular flicking. Using direct mechanical stimulation of identified individual feathered sensilla, flagellar flicks were consistently evoked in a small percentage of the preparations we examined.

Our findings suggest that, although it is sometimes possible to trigger the flick reflex by stimulating a single standing feathered sensillum, the most effective stimuli, at least in terms of minimum response latency, are those that affect the entire array of feathered and, perhaps, other sensilla on the lateral flagellum. This conclusion is inferred first from the fact that, following a mechanical stimulus to the base of the flagellum, initial spikes from most or all of the standing feathered sensilla arrive at the brain within a 5 ms time window (Mellon, 2010), probably as a consequence of the position-dependent changes in flagellar axonal conduction velocity discovered in earlier studies (Mellon and Christison-Lagay, 2008; Mellon, 2010). The basis for the large latency differences in the flick reflex following stimulation of an individual sensillum and the entire flagellum must therefore depend largely upon afferent recruitment and central summation, respectively, with the shortest response latencies observed presumably after simultaneous stimulation of the entire array of feathered sensilla. This conclusion is supported by our observations of increased response latencies in truncated flagella compared with intact flagella. Furthermore, because the movement of the flagellum base that is due to the stimulus should be the same or even more vigorous with the loss of its distal mass, it is hard to reconcile the findings of increased response latency with the possibility that chordotonal joint receptors in the basal three segments are responsible for the evoked flicks.

Working with the marine hermit crab *Pagurus alaskensis*, Snow (Snow, 1975b) found that removing segments from the lateral antennular flagellum effectively reduced the number of spikes per burst in one of the motor neurons driving flicking in that animal. We measured both spike number and frequency in motor neuron bursts in the preparations with truncated antennules that we examined, but we found no consistent difference in these parameters from those measured in the respective intact flagella. As an additional difference between the crayfish and hermit crab, the return stroke during antennular flicking in *P. alaskensis* is accomplished through an extensor muscle and is triggered by proprioceptors in joints at the base of the antennule (Snow, 1975b), again in contrast to the passive return mechanism in *Procambarus*.

The finding that selected movement of individual feathered sensilla can trigger flicking in some preparations is indicative of the sensitive central tuning of this behavior and provides some indication of its importance to the animal. At the same time, these observations raise interesting questions about the physiological basis for the reflex. Although in most cases stimulation of individual feathered sensilla was not sufficient to evoke reflex flicking, in two preparations the sensilla responses to individual stimulation were sufficiently robust and/or their central targets were sufficiently sensitive for the reflex to be triggered. Nonetheless, it remains unclear how the reflex could be evoked by stimulation of just the one or two sensory neurons associated with a single feathered sensillum, against the background of frequent random spiking generated by the entire array of sensilla. Even though this is not the only arthropod sensillum-triggered reflex behavior evoked by activation of one or two sensory axons (Lewis, 1953; Grabowski and Dethier, 1954), it occurs against the background of considerable sensory noise. Although the explanation for this conceptual problem is presently unknown, one possibility is that direct electrical connections between sensillar afferent terminals amplify the amount of synaptic transmitter released by spikes that arrive at the brain over an axonal pathway. This in fact is already known to be a mechanism that can enhance the postsynaptic response of crayfish lateral giant neurons in the abdomen to synchronized, hydrodynamic afferent inputs (Edwards et al., 1998). Another possible mechanism would be robust synaptic facilitation at the central terminals of sensillar axons within the deutocerebrum, thereby generating the required suprathreshold input depolarization following the arrival of high-frequency spiking in a single input pathway. That being said, it is clear that temporal summation among the several feathered sensillar pathways usually appears to be required to trigger the reflex in most of the preparations we examined, and it is arguably responsible for the minimum response latencies observed (30–40 ms) following global stimulation of the flagellum.

A different question concerns the reafference that must be generated by flicking itself: how is this behavior not self-regenerative? Again, there is no immediate answer, but reafference may be prevented through feed-forward inhibition of the afferent terminals during the flick, a mechanism known to effectively prevent multiple tailflips following activation of the lateral giant axons in the crayfish abdominal nerve cord (Kennedy et al., 1974). As has been suggested previously (Mellon and Christison-Lagay, 2008), standing feathered sensilla of the crayfish antennules are also implicated in triggering tailflip startle responses mediated by the medial giant fibers, and a feed-forward inhibitory mechanism may also prevent spurious tailflips evoked by antennular flicking. It is clear in any event that powerful central suppression of near-field inputs must occur during both of these evoked behaviors.

Finally, assuming that standing feathered sensilla are indeed involved in triggering medial giant fiber-mediated startle responses

as well as antennular flicking, what are the characteristics of the neural code that trigger the latter and not the former? It is possible, given our current findings, that flicking will be triggered by slight hydrodynamic disturbances, such as small eddies, which activate only a few (and perhaps just one) of the feathered sensilla arrayed along the lateral flagellum, whereas an approaching predator would be expected to generate a strong leading wavefront that could impact the entire antennule, including the feathered sensilla on the medial flagellum. This question, like those discussed above, will have to be addressed at the level of the central targets of these sensors in the lateral and medial antennular neuropils.

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