

## Not-so-simple setae

I write to express my serious reservations concerning some of the scientific conclusions and statements of attribution made in a recently published account by Monteclaro et al. in *The Journal of Experimental Biology*, entitled ‘Response properties of crayfish antennules to hydrodynamic stimuli: functional differences in the lateral and medial flagella’ (Monteclaro et al., 2010). Among the most apparent problems with this study is the misidentification of the sensillar class responding to hydrodynamic stimulation. Despite the title of this paper, its major thrust is to implicate a class of setae on the two antennular flagella as hydrodynamic receptors and to measure their spiking responses and thresholds to sinusoidal disturbances in the surrounding fluid column. The setal class so identified as ‘simple’ setae are identical to those my late colleague J. A. C. Humphrey and I previously described (Mellon and Humphrey, 2007; Humphrey and Mellon, 2007) as ‘beaked’ sensilla. From very preliminary data I have obtained, it is probable that this class of setae represents bimodal mechano-chemoreceptive contact sensilla, as found on the first antennae and walking appendages of other decapod crustaceans (e.g. Cate and Derby, 2002; Schmidt and Derby, 2005). What seems abundantly clear, however, is that they are not the hydrodynamic-responsive setae from which Monteclaro et al. (Monteclaro et al., 2010) were recording in their study. I draw this conclusion from the following points of argument.

First and foremost, in recordings from multiunit nerve tracts, one cannot draw conclusions as to the source of a population of spikes unless one obtains positive correlation with observed sensillar movement and the resultant activity. Monteclaro et al. (Monteclaro et al., 2010), according to a reasonable interpretation of their methods, apparently did not perform this crucial, meticulous visual identification step to determine the sensillar origins of the spiking axons in response to mechanical manipulation. It would have been necessary to use suitably small probes mounted on a micromanipulator to determine the precise setal origin of the activity corresponding to the applied stimulus. If this had been done with suitably high magnification (100×), the authors would have determined that the large spiking responses they obtained only occur following movement of standing feathered (a.k.a. plumose) sensilla or the few very long filamentous sensilla that are sparsely distributed along both antennular flagella. Unlike the far more numerous simple setae incorrectly identified as the source of this activity by the authors, the attachment socket of the standing feathered sensilla with the flagellar cuticle has the requisite high compliance to be extremely sensitive to even minute fluid motions in their surroundings. Moreover, we have found that mechanical probing of simple setae will, due to their socket stiffness, cause the entire flagellum to be displaced, thereby exciting axons actually originating in the standing feathered sensilla. This may be the basis for the authors’ mistaken identification of the origin of the activity following hydrodynamic stimulation.

In our experience from recording spiking activity in axons originating from several hundred sensilla on both flagella (Mellon and Christison-Lagay, 2008; Mellon, 2010), we have never observed electrical responses to mechanical stimulation of simple setae that were not buried in the noise level (10–50 μV). Adequately high input-impedance electrodes and/or techniques (e.g. subdividing the nerve into very small strands) would be required to resolve the tiny electrical signals emanating from the small-diameter axons innervating the simple setae, not the 40 to 70 μm comparatively low-resistance suction electrodes utilized by the authors. Large spikes in the study by Monteclaro et al. (Monteclaro et al., 2010), reported as up to 10 mV in amplitude and having a high signal-to-noise ratio, are undoubtedly from plumose standing sensilla, in agreement with our own findings

using similar low input-impedance recording techniques (Mellon and Christison-Lagay, 2008).

The ablation experiments reported in Monteclaro et al. (Monteclaro et al., 2010) are unfortunately also imprecisely described and documented. What methods were used to ablate and/or shave the flagella? To which seta class are the authors referring when they state, “Sinusoidal stimulation of seta-less lateral and medial flagella did not induce any response” (p. 3686, right column)? Importantly, where are the electrical records to document their statements? Perhaps most critical is the statement on p. 3685 that cutting a “...large part of the proximal half [of the flagellum]...” was used as a technique to remove standing plumose sensilla. This procedure may remove most of the procumbent setae, which are non-innervated and thus non-responsive in any event, but will remove little more than one-half of the population of standing plumose sensilla [fig. 1C in Mellon and Christison-Lagay (Mellon and Christison-Lagay, 2008)]. It is not surprising, therefore, that the authors obtained responses to hydrodynamic stimulation from flagella so treated, and obtained threshold data for units that were similar to those obtained from untreated flagella. (The text does not say whether the data in fig. 4 represent spikes from a single sensillar axon or from many more, a crucial point in experiments where an entire setal class is said to be absent. How did the authors determine that a single standing plumose sensillum did not remain?)

Finally, the authors state on p. 3684 of their article that, in our 2007 papers, Humphrey and I reported that the simple setae are responsible for the asymmetrical responses of the deutocerebral neurons to hydrodynamic inputs. Although we did observe that the simple setae were the most numerous putative sensilla on the lateral flagellum, and were a possible source of near-field input, we never identified them *per se* as the afferent pathway for hydrodynamic inputs to deutocerebral interneurons (Mellon and Humphrey, 2007). Moreover, our use of simple setae in the companion theoretical paper (Humphrey and Mellon, 2007) was as semi-rigid, convenient model setae to examine fluid mechanics in the vicinity of the lateral flagellum, not to infer that these structures were identified as hydrodynamic receptors.

This correspondence is written as an effort to clarify within the literature what I believe to be some erroneous published conclusions and statements concerning the sensory physiology of the crayfish antennule.

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## Response to 'Not-so-simple setae'

In his Correspondence article (p. 871), DeForest Mellon, Jr argues that the type of seta in our report (Monteclaro et al., 2010) was erroneously identified. In response, we would like to make the following remarks.

Stimulation of an antennule that was either seta-less or without plumose setae was performed by shaving and cutting setae under the microscope. Cognizant of the fact that a few plumose setae are present in the distal half of the flagellum, we shaved and cut the remaining plumose setae after cutting the proximal half to remove a large proportion of procumbent and standing plumose setae. Examination of these flagella using SEM revealed the presence of medium simple setae and long simple setae on both flagella, approximately two to six procumbent plumose setae on each flagellum, and aesthetascs and associated setae on the lateral flagella. Fig. 1 shows recordings from a seta-less (i.e. all setae removed) medial flagellum during sinusoidal stimulation. Stimulation failed to elicit a response from the flagella, suggesting that the increase in spike discharges resulted from deflection of setal shaft.

Our work did not detail the physical and ultrastructural organization of putative mechanoreceptors. In identifying mechanoreceptive afferents in the crayfish antennules, we depended largely on published literature that reported mechanosensitivity of antennular setae (Chichibu et al., 1978a; Chichibu et al., 1978b; Humphrey and Mellon, 2007; Mellon and Humphrey, 2007; Mellon and Christison-Lagay, 2008). Although we did describe the medium simple setae to be the most dominant setal type present on the dorsal half of both lateral and medial flagella, we did not specifically identify this setal class as the receptor with neurons that responded to our sinusoidal stimulation. In fact, the same impression can be gathered in the papers of Mellon and Humphrey (Mellon and Humphrey, 2007) and Humphrey and Mellon (Humphrey and Mellon, 2007) on the hydrodynamic responses of

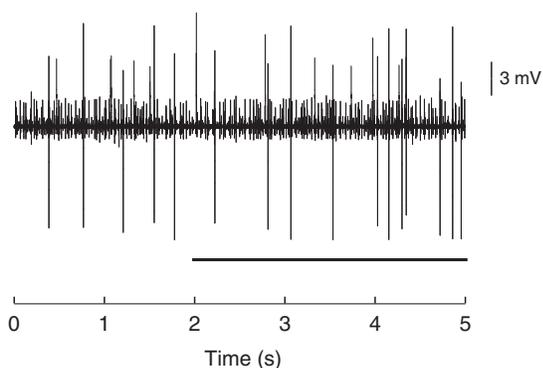


Fig. 1. Response of mechanoreceptor neurons in a seta-less medial flagellum when stimulated sinusoidally at 10 Hz with water velocity  $26.7 \mu\text{m s}^{-1}$  r.m.s. Horizontal line indicates duration of the stimulus.

neurons in the crayfish antennules to fluid flow, and their reference to simple setae as the most numerous setal type in the lateral flagellum (although our experience showed that this distinction belongs to the procumbent plumose setae). Similarly, these authors also stopped short of identifying medium simple setae as the actual receptor in the fluid dynamics test.

We do esteem and appreciate the comments of Dr Mellon, who has worked a great deal on the subject of crayfish sensory transduction. Our recording technique, i.e. the use of suction electrodes, required us to remove the carapace on the proximal aspect of the flagellum and, consequently, the aggregation of large standing feathered setae that are present in the same area. The removal of these large setae would leave the relatively smaller-sized standing plumose setae on the other segments of the flagellum. Whether these smaller-sized standing plumose setae would respond similarly to the larger-sized counterparts has yet to be reported. In addition, a comparison of the mechanosensitivity of all three putative mechanoreceptor setae – medium simple setae, long simple setae and standing plumose setae – has yet to be shown. Notwithstanding the absence of the actual identity of specific neurons that responded to the mechanical stimulation, we feel that this does not affect any of the conclusions of our article, such as the general mechanosensitivity of both lateral and medial flagella, the relevance of the mechanoreceptors on the antennules during antennular depression, or our attempt to compare crustacean mechanosensitivity with that of the fish mechanosensory system.

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