PHYSICAL PROPERTIES, SENSORY RECEPTORS AND TACTILE REFLEXES OF THE ANTENNA OF THE AUSTRALIAN FRESHWATER CRAYFISH *CHERAX DESTRUCTOR*

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Summary

1. The antennal flagellum of the crayfish *Cherax destructor* is very flexible, tapered and is almost as long as the body in mature animals. It consists of a series of short segments, oval in cross-section, that take the form of flattened rings at the base and slender tubes at the tip. There are no muscles in the flagellum.

2. The flagellum's mechanical resistance to being bent is not the same in all directions. It is most easily bent dorsally and least easily bent medially, when held stretched out horizontally in front of the animal. The resistance to bending is 10 times less than that of *Astacus*.

3. The individual segments of the flagellum form curved articulating surfaces that may assist in reducing torsional movement of the flagellum when it is bent and, together with the tapered form, provide the flagellum with unique mechanical properties.

4. Four main types of hairs are found on the flagellum. They are distributed in a specific manner, and total about 7000 hairs on the flagellum of a mature animal. Cross-sections through the sensory nerve reveal only about 2000 axons. Methylene blue applied to fresh preparations stains dendrites to all smooth hairs but never to procumbent feathered hairs.

5. Physiological recordings from the sensory axons of the flagellar nerve allowed the characterization of the sensory hair response to displacement. Recordings were also made from slowly adapting receptors, sensitive to bending the flagellum in particular directions, but not related to mechanoreceptive hairs.

6. Intracellular recordings from identified extensor and flexor motoneurones revealed the ordered projection of the inputs from the receptor hairs, and also provided a model that correlates specific receptor inputs with particular antennal reflexes, and links the unique mechanical properties of the flagellum with the detection of the distance of near objects.

Introduction

Arthropod antennae are characterized by their sensitivity to mechanical

displacement and chemical stimuli. In decapod crustaceans these two modalities have been separated to some extent, the first antennae or antennules taking over the main chemoreceptive function and the second antennae being predominantly tactile. Recent behavioural studies of freshwater crayfish have shown that the antennae can be used to detect the position relative to the animal's body of both a distant vibrating object (Tautz, 1987; Tautz & Hamm, 1986; Tautz *et al.* 1981; Masters *et al.* 1982) and a tactile stimulus applied directly to the flagellum (Zeil *et al.* 1985; Sandeman & Varju, 1988). The role played by the antennae in active animals is not yet understood. Active antennal movements are large, not always bilaterally coordinated and difficult to quantify (Zeil *et al.* 1985). It is not known if animals can use active antennal movements to extract spatial information about objects near them, or whether the movements serve only to establish the distance between the animal and such objects more accurately than the visual system can. To answer such questions about the role of antennae we need to know more about their mechanical properties and the responses of the receptors under conditions similar to those imposed on them during active antennal movements. A consideration of the tactile reflexes triggered by these receptors can be exploited to provide information about the sensory/motor connectivity of the system.

This paper reports the results of a study of the antennal flagellum of the crayfish *Cherax*. It was found that the flagella of *Cherax* are about 10 times more flexible than those of *Cambarus* and *Astacus* (Taylor, 1975; Masters *et al.* 1982). Recordings were made from slowly adapting receptors that monitor bending of the flagellum along different radii and physiological evidence indicated that these receptors are not associated with the flagellar hairs. Counts of hairs on the flagellum and of axons in the flagellar nerve of *Cherax* indicated that, as in *Astacus* (Bender *et al.* 1984), the procumbent feather hairs are not innervated. Intracellular recordings from identified tonic extensor and flexor motoneurones in the antennal neuropile showed that the various flagellar receptor systems are addressed to different motor systems according to their position on the flagellum and produce both excitation and inhibition in these motoneurones.

Materials and methods

Crayfish were obtained from ponds in the Sydney area and kept in aquaria in the laboratory.

Physical properties

Dimensions of flagellar segments were measured microscopically.

Measurements of the flagellum's resistance to lateral displacement were made on fresh, isolated flagella, clamped at their bases and submerged in crayfish saline. A flagellum was threaded through a small hole in a thin metal plate and an Akers 180 resistive strain gauge brought to bear on the flagellum 5 mm from the plate. The flagellum was then displaced through 1 mm with the strain gauge and the resulting change in potential from the strain gauge recorded (Fig. 1A). The strain
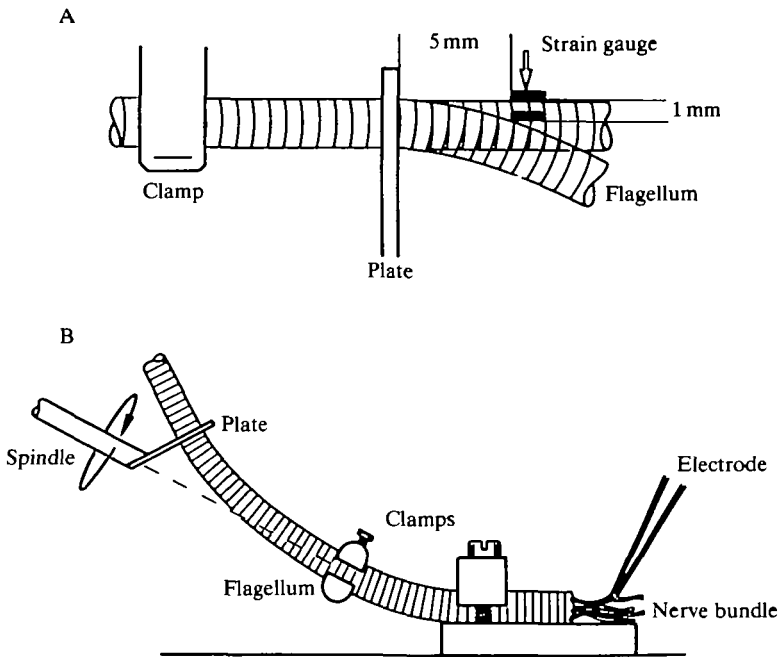
A

Fig. 1. (A) To obtain a measure of stiffness, the flagellum was clamped at its proximal end and the distal portion was passed through a hole in a metal plate. The beam of a strain gauge was brought to bear on the flagellum 5 mm from the plate, and used to force the flagellum 1 mm to the side. The resulting voltage change was recorded. The process was repeated at 5 mm intervals along the flagellum. (B) Electrophysiological recordings from the sensory nerves at the base of the flagellum were made with a suction electrode. The flagellum was clamped near its base so as to direct the distal end upwards towards a plate mounted eccentrically on the spindle of a servo-motor. The distal end of the flagellum was passed through a hole in the plate. The distance from the clamp to the plate was 5 mm and the lateral displacement of the antenna 1 mm. Rotation of the plate displaced the flagellum by an equal amount in all directions.

gauge response of 1 mV/50 mg load was linear over the range 1–20 mV and all measurements taken fell within this range. Maximum displacement of the strain gauge beam was less than 5 μm, or 0·5 % of the flagellar displacement, and was ignored. The resistance of the flagellum to displacement is expressed as torque in Nm rad^{-1}.

Receptor anatomy

Antennal flagella from freshly moulted animals were prepared for scanning electron microscopy by fixing in 5 % glutaraldehyde, washing, dehydrating and then air drying from xylene in a dust-free chamber over 2 days. The dried flagella were glued to stubs, coated and viewed. Methylene blue preparations were made by slitting a flagellum longitudinally with a sharp scalpel and immersing the two halves in a dilute solution of methylene blue. Owing to the poor penetrating

properties of the stain, this method could not be relied upon to stain all the receptors. However, after slitting the flagellum open, the inner tissue could be stripped out of the flagellum and pinned alongside the piece of flagellum from which it came. Pigment bands marked the boundary of each segment so that a direct comparison could be made between the numbers of hairs on the outer surface of the flagellum and the receptor cells that lay beneath them. Staining such preparations in methylene blue gave a better indication of the true complexity of the sensory system in the flagellum. Permanent preparations were made by pinning out the stained preparations on wax, prefixing in 5 % glutaraldehyde for 60 s and fixing in 12 % ammonium molybdate overnight. The preparations were washed for 10 min in phosphate buffer, removed from the wax, gently pressed flat onto a microscope slide and air dried. They were then covered with a layer of molten 3 % Noble agar (Difco). When this had cooled, the preparations were dehydrated for 20 min in absolute ethanol, cleared in methyl salicylate and mounted in DePeX (Gurr). The Noble agar coating fixed the preparation to the slide and prevented severe distortion of the tissue during the rapid dehydration and clearing.

Receptor physiology

A fresh, isolated flagellum was clamped at its proximal end to a plate. A second clamp served to deflect the distal end upwards where it was passed through a hole in a metal plate, fixed eccentrically to the spindle of a servo-motor. The entire flagellum was submerged in crayfish saline and a length of sensory nerve exposed at the proximal end. A suction electrode was applied to the exposed nerve to record the action potentials of the flagellar receptors. The source of single unit discharges was traced by running the bristles of a paint brush along the flagellum to locate their general area and then stimulating the hairs singly with a bristle under microscopic observation. Constant velocity (180 degrees s^{-1}) rotation of the eccentric plate resulted in the flagellum being bent laterally in all directions about its long axis for each complete rotation of the spindle (Fig. 1B). Rotation of the flagellum was monitored potentiometrically and displayed simultaneously with the single receptor units on an x–y plotter.

Motoneurone physiology

Semi-isolated, perfused brain preparations were used for these studies. Before exposing the brain, fine wire electrodes, insulated except at their tips, were pushed through a small hole in the cuticle and cemented in place in tonic flexor or extensor muscles in the antennal carpopodite. The head of the animal was rapidly removed, clamped in a stand and perfused through the dorsal aorta with cold saline under pressure. The area over the antennal lobe was desheathed and the motoneurone corresponding to the unit recorded in the muscle found by prospecting. The antenna was held firmly at its base by a small clamp that fitted over the joint
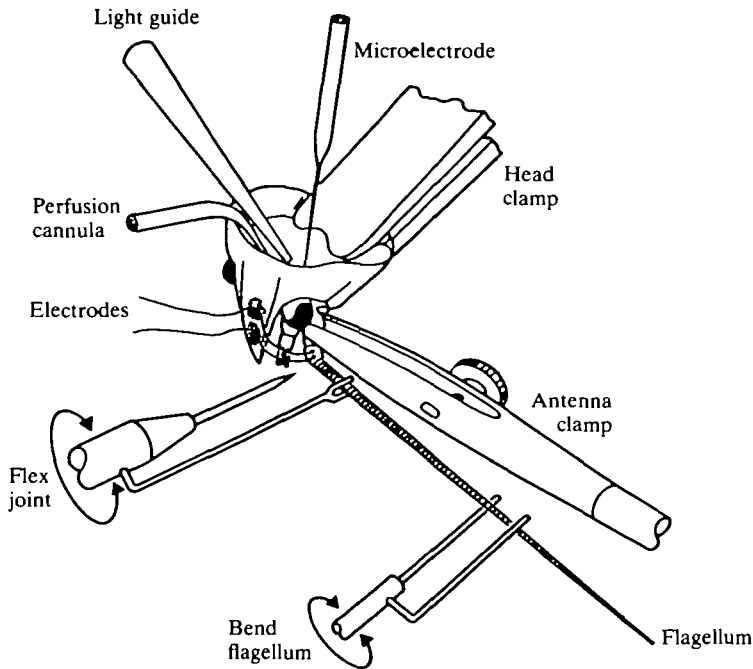
Fig. 2. Simultaneous recordings from motoneurones in the brain and from the muscles they innervate in the antenna were made using a semi-isolated brain preparation. After inserting the extracellular electrodes in the antennal carpopodite, the head of the animal was rapidly removed from the body and clamped rostrum down. The cerebral artery was perfused with cold crayfish saline. The ischiopodite/meropodite joint was clamped without impeding the lateral movements of the flagellum about the carpo-podite. The flagellum was held near its base in a small fork, mounted eccentrically on the spindle of a servo-motor. A pointer on the spindle allowed its centre of rotation to be aligned with the flagellum/carpopodite joint. Rotation of the spindle flexed this joint and stimulated the chordotonal organ within it. Bending the more distal portion of the flagellum in a lateral or medial direction was achieved with a second pair of levers, mounted on the spindle of a potentiometer and moved by hand. Central recordings were made with intracellular microelectrodes. Illumination of the brain with a light guide allowed the axon tracts and the cell somata to be visualized, facilitating accurate placement of the electrode tip.

between the ischiopodite and meropodite, allowing the flagellum to project to the side and to articulate freely about the carpopodite (Fig. 2).

Three stimuli were applied. (1) The flagellum was stroked along its medial or lateral edge with a small paint brush. (2) The flagellum was deflected either medially or laterally about its joint with the carpopodite using a small fork mounted eccentrically on the spindle of a servo-motor. (3) The more distal portion of the flagellum was bent either medially or laterally with a small lever mounted on the spindle of a potentiometer, while the more proximal part of the flagellum and the flagellum/carpopodite joint were held stationary (Fig. 2).
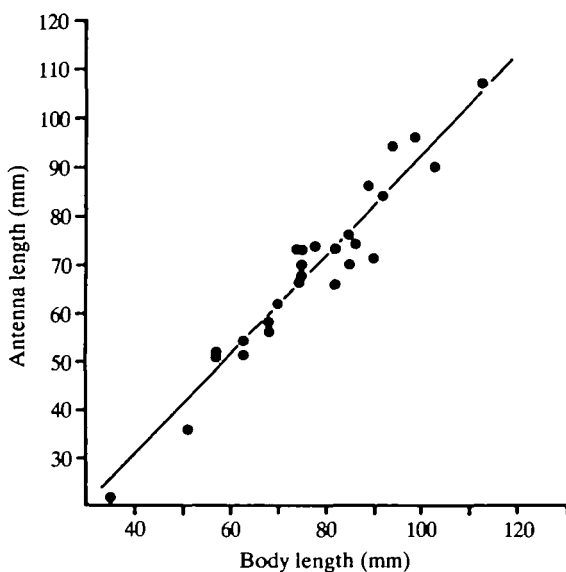
Fig. 3. The relationship between the length of the body (rostrum to telson) and the length of the flagellum. In the largest animals the flagellum is only 6 % shorter than the body; smaller animals have antennae up to 40 % shorter than the body. The line drawn through the points is a linear regression with a correlation coefficient of 0·9575.

Results

Mechanical properties

Dimensions

Cherax destructor reaches a maximum size of about 120 mm. In small individuals the flagella could be up to 40 % shorter than the body length whereas those of the larger animals were only 6 % shorter than the body length (Fig. 3).

The antennal flagellum of *Cherax* is made up of a series of annular segments linked to each other by arthrodial membranes. *Cherax* flagella that were 55–90 mm long had between 220 and 250 segments. These segments were oval in cross-section and the ratio of the major to the minor diameters was 1·4:1. This ratio was maintained along the length of the flagellum. The average diameter of the segments, calculated from the means of the radii of major and minor cross-sectional axes, decreased linearly from the base to the tip (Fig. 4B). The length of the segments, however, increased from the base to tip (Fig. 4A). Thus the segments take the approximate form of flattened rings at the base and slender tubes at the tip.

Mechanical resistance

The resistance of the *Cherax* flagellum to bending varied with the direction of the displacement in relation to its long axis. In intact animals, flagella that point directly forwards were least resistant to upward displacement and most resistant to

displacement towards the midline. Values for resistance to displacement down-
wards and to the side lay between these extremes (Fig. 5). The resistance of the
Cherax flagellum decreased exponentially when displaced laterally or dorsally. It
was less resistant to being bent along the minor axis than along the major axis
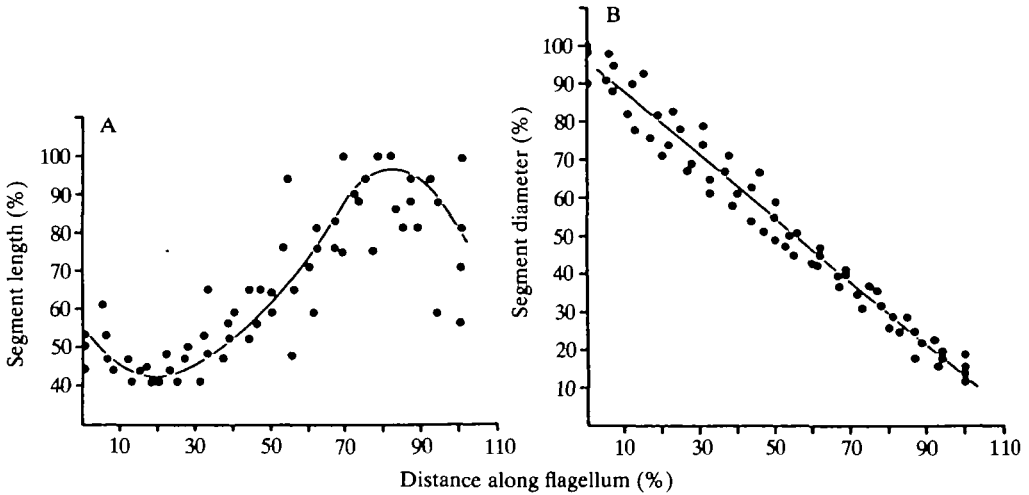(Fig. 6). Typical values for the mechanical resistance of the proximal part of the

Fig. 4. (A) The change in the length of segments along the flagellum from base to tip.
Segment length is plotted as a percentage of that of the longest segment against the
distance along the flagellum. The data are pooled from five animals with flagella
ranging from 55 to 90 mm long and the distance along the flagellum normalized as a
percentage of the total length. The curve was fitted by eye to the means of points falling
within 5 % sections along the flagellum. (B) The decrease in average diameter of
segments along the flagellum from base to tip. Segment diameter (taken as the mean of
major and minor diameters) is plotted as a percentage of that of the broadest segment.
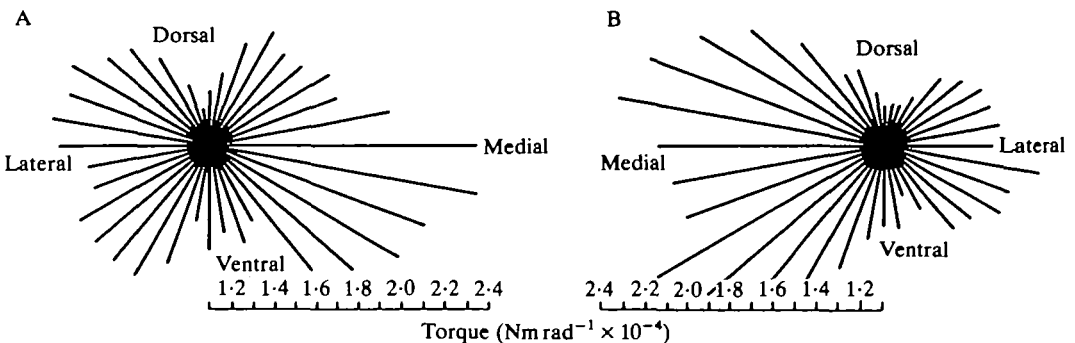The data are from the same five animals used in A.

Fig. 5. Mechanical resistance of the proximal portion of the right (A) and left (B)
flagella to being bent, measured as the torque exerted by a flagellum when forced along
different radii through 11·3° of arc. The lines represent the resistance of the flagellum
to being bent along the different radii according to the scale. The flagellum is more
resistant to being bent medially than laterally. It is most easily bent dorsally.

flagellum in *Cherax* were 0·001–0·0014 Nm rad^{-1}. These are 10 times less than those obtained for *Astacus* (Masters *et al.* 1982).

Articulation

When the tip of a *Cherax* flagellum is forced against an obstruction, as it often is in active animals, it behaves in a unique way. The flagellum bends easily but, perhaps more significantly, the bent portion of the flagellum is confined to relatively few segments close to the obstruction. As the obstruction is moved towards the animal, so the bent portion of the flagellum 'rolls' down towards the antennal base, the distal part of it lying flat along the advancing surface (Fig. 7A). Eventually the flagellum's resistance to bending overcomes the frictional force between the distal portion of the flagellum and the object, and the flagellum slides past the obstruction and re-assumes its straightened form. Consistent with the different relative stiffness to lateral and medial bending, the flagellum can be bent through a greater angle outwards than inwards, before it slides past an obstruction (Fig. 7B). Because the tracings in Fig. 7A,B are from an isolated antenna, the behaviour of the flagellum cannot be due to any muscular effort on the part of the animal, but are inherent in the mechanics of the articulating segments.

Closer examination of the flagellar segments revealed that each has shaped articulating surfaces that fit into the preceding and following segments (Fig. 8A). When the flagellum is bent, the arthrodial membrane on the inner side of the bend is rolled inwards, a factor which may be significant in signalling displacement of the joint *via* mechanoreceptive hairs (see below).
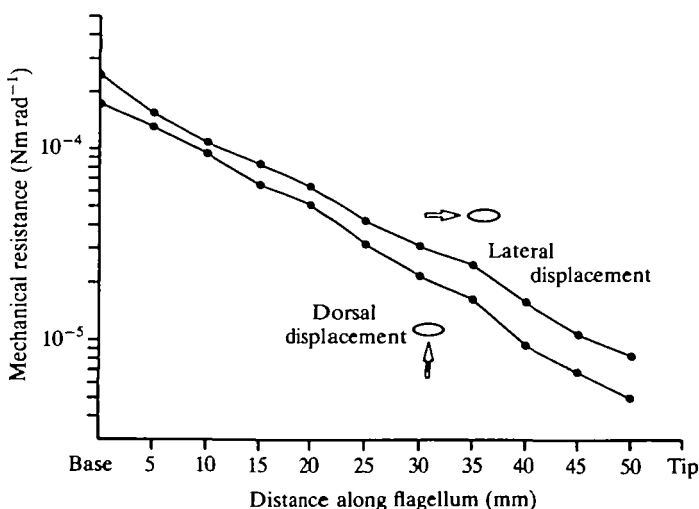

Fig. 6. The mechanical resistance of a flagellum to being bent at different points along its length, measured as the torque required to bend it through 11·3° of arc. The resistance decreases exponentially along the length of the flagellum.

The sensory receptors

Receptor types

There are four hair types projecting from the surface of the flagellum of *Cherax*. (1) Feathered procumbent hairs that insert around the distal edges of each
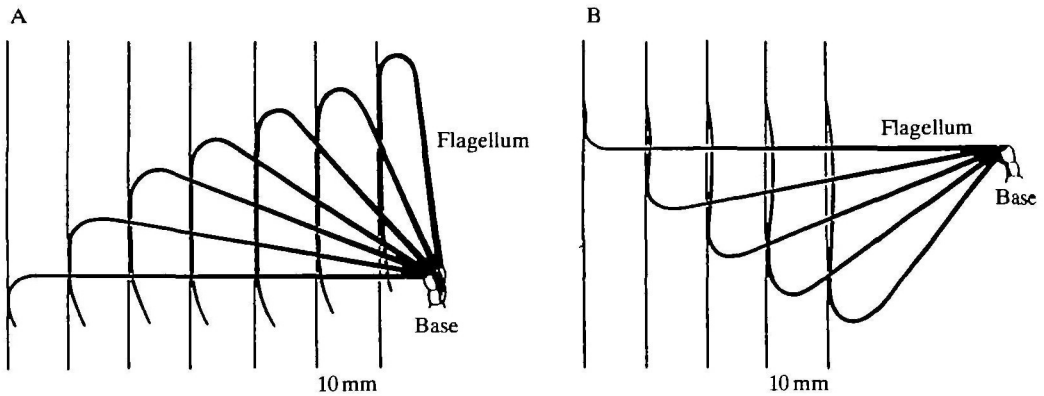

Fig. 7. Bringing a flat surface against an isolated flagellum shows how the bent portion of the flagellum 'rolls' towards its base while remaining close to the point of contact with the obstruction. (A) Bent laterally the flagellum is almost doubled back on itself before sliding past the obstruction. (B) Medially bent, the flagellum slides past the obstruction sooner because of the greater resistance to being bent in this direction. The tracings were obtained from freshly isolated antenna, clamped so that the joints between the flagellum, propodite and carpopodite were all free to move.
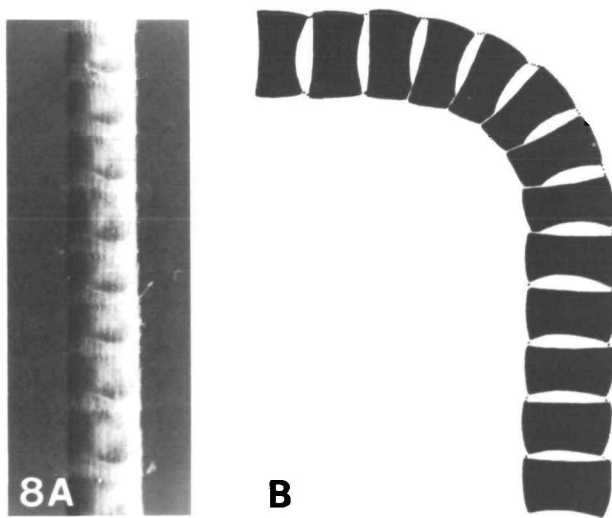

Fig. 8. (A) A photograph taken obliquely of the flagellum to show its ventral and lateral surfaces, and the near sinusoidal form of the articulating edges of the flagellar segments. ×10. (B) A tracing of a photograph of a flagellum bent laterally to show how the articulating surfaces on the inside of the bend roll into one another.

segment, project across the arthrodial membrane between two segments and lie closely pressed against the next segment (Fig. 9A–E).

(2) Short smooth hairs that project outwards from the flagellum. At first sight these appeared all to belong to a single class, but closer examination showed three distinct subtypes, characterized by differences at their tips. They often appeared together in triplets on the medial edges of the flagellum. All three are simple and without lateral branches. They all appear to be hollow and might open at the tip. The tips are sharp, blunt or bifid (Fig. 9G–J). The short smooth hairs resisted deflection towards the base of the flagellum, but hinged smoothly forwards to lie flush against the flagellum. The hairs in fresh preparations were not displaced by movements of the bathing Ringer's solution. As a group they are probably homologous with smooth upright hairs identified in *Astacus* (Tautz *et al.* 1981) and to type B smooth hairs found in *Homarus* (Tazaki, 1977).

(3) Long smooth upright hairs that sit in characteristically large sockets and end in smoothly pointed tips with some fine branches extending outwards at the tip. They have a constriction near the base giving them a segmented appearance (Fig. 9G–I). These hairs are very mobile and were affected by the slightest movement of the bathing medium. They are homologous with type A hairs in *Homarus* and in *Astacus* (Tautz, 1981; Tazaki, 1977).

(4) Short round-ended peg hairs, unique to *Cherax* (Fig. 9E,F,J).
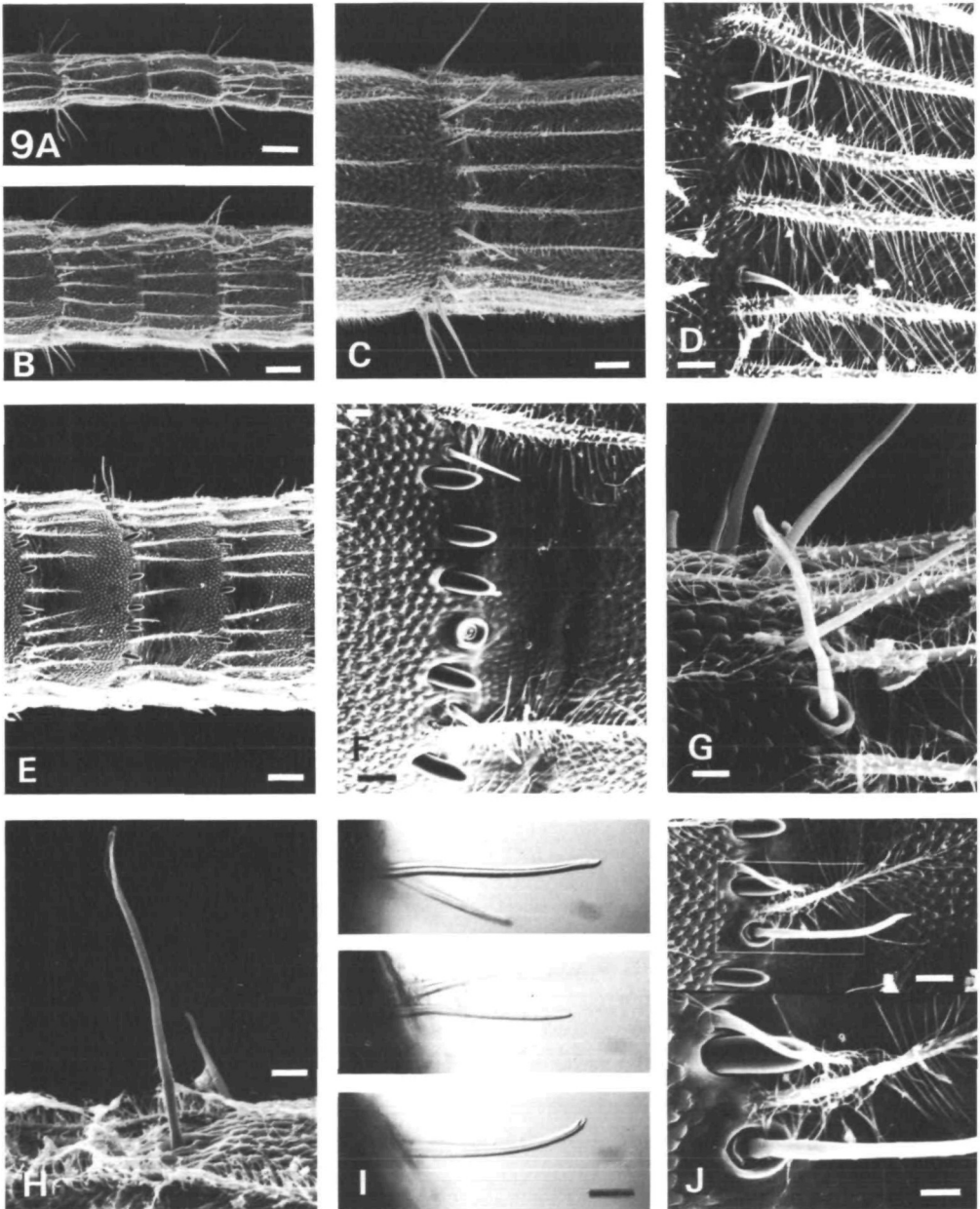
Distribution

There are 30–40 receptor hairs on each segment and about 7300 receptor hairs in total on flagella containing more than 200 segments. The distribution of the hair types is not uniform along the length of the flagellum. A flagellum of 70 mm (219 segments) carried a total of 4020 feather hairs, 2706 short and long smooth hairs and 624 peg hairs. Of the smooth hairs only about 200 were long. The distribution of the feather hairs around any segment was fairly uniform. 18 % of the smooth hairs (all kinds) were found on the dorsal surface, 19 % on the ventral surface, 40 % on the medial surface and 23 % on the lateral surface.

Fig. 9. Scanning electron micrographs and photomicrographs that show the distribution and types of hair found on the *Cherax* flagellum. The smooth hairs near the tip of the flagellum project from alternate segments (A,B), whereas the procumbent feathered hairs are found on every segment and lie flat against the segment distal to the one on which they arise (C). The procumbent feather hairs are not all associated with smooth hairs (D). Half-way along the flagellum, the feather hairs on the dorsal and ventral surfaces of the flagellum give way to the short peg hairs (E,F). Long smooth hairs have fine filaments extending from their tips and have their bases in a large cup-shaped socket (G,H). The short smooth hairs appear to be hollow and can be divided into three sub-types according to whether the tip is blunt, sharp or bifid (I) (Nomarski optics, fresh tissue). The three sub-types of short smooth hair are often found together in triplets on the medial edges of the flagellum. All four hair types (long smooth, short smooth, feather and peg) can be found close to each other on some areas of the flagellum (J). Calibration bars, A,B,E, 100 μm; C, 50 μm; D,F,I,J(upper), 25 μm; G,H,J(lower), 12·5 μm.

The peg hairs were distributed over the middle three-fifths of the flagellum and more were found on the ventral than on the dorsal surface. Fewer were found on the lateral or medial edges (Fig. 10).

Innervation

Procumbent feathered hairs on the flagellum of *Astacus* are not innervated (Bender *et al.* 1984). Methylene blue vital staining of *Cherax* flagella showed primary receptor cells that were always associated with the smooth hairs and never
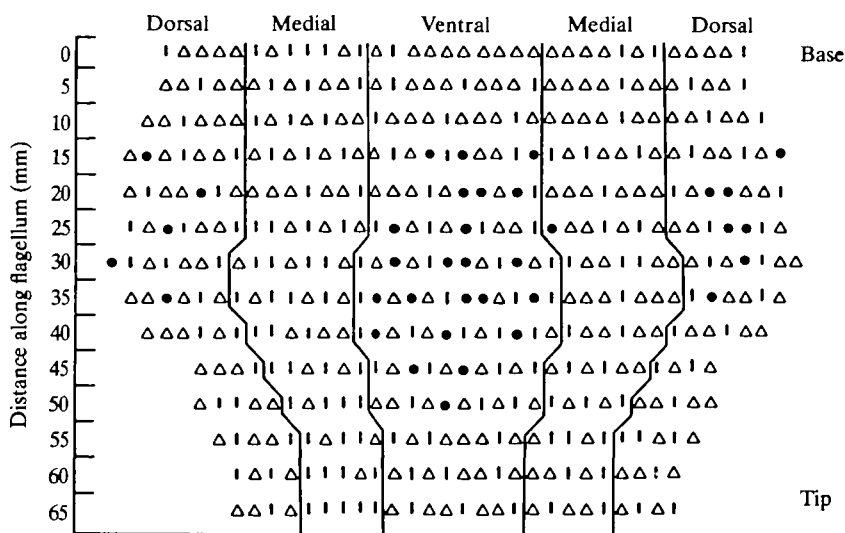
Fig. 10. The distribution of the hair types along a flagellum, sampled at 5 mm intervals from base to tip. Smooth hairs (I) predominate along the medial edge. Peg hairs (●) are concentrated on the ventral and dorsal surfaces of the middle portion of the flagellum. Many of the procumbent feather hairs (△) are not paired with smooth hairs.

seen to insert their dendrites at the bases of the feathered hairs. Between 1600 and 2300 axons were counted in toluidene blue-stained, 1 μm sections of the proximal ends of the flagellar nerves of three antennae. Electron micrographs of individual bundles of the flagellar nerve revealed no very fine axons that would have been missed in light microscope counts. The axon counts were less than one-third of the total number of hairs on a flagellum. Considering that many of the smooth hairs in *Cherax* appeared to be multiply innervated, I conclude that, as in *Astacus*, the feather hairs in *Cherax* are not innervated, although final confirmation can only come from an ultrastructural examination of the bases of these hairs as has been done for *Astacus* (Bender *et al.* 1984).

Physiology

The responses of the two types of smooth hairs to mechanical stimulation was very consistent.

Long smooth hairs responded with short phasic bursts to movements of the bathing medium and to rotation of the flagellum. Plucking the hairs off abolished their response to water movement.

Short smooth hairs were insensitive to water movements even when these were fairly gross. Their responses were always rapid phasic bursts to inward or outward deflections of the individual hair. In some cases, rotation of the flagellum led to the discharge of these units. Bending the flagellum towards the hairs stimulated them, but they adapted rapidly to a maintained deflection of the flagellum. Plucking the short smooth hairs off did not always abolish the response to antennal deflection.

This was especially noticeable if the smooth hair was located around the lateral or medial edge of the flagellum, where the arthrodial membrane was rolled inwards during flagellar deflection. Complete removal of feathered hairs next to or in the vicinity of a smooth hair never led to, or altered, its receptor discharge. No evidence was found of a mechanical coupling between feathered hairs and smooth hairs as in *Astacus* (Bender *et al.* 1984). No responses to mechanical stimuli were ever traced to the feather hairs or the peg hairs.

Many recordings were made of the action potentials from receptors that responded to deflection of the flagellum but not to stimulation of receptor hairs. The responses from these receptors were characterized by their directionality, and slow adaptation to maintained deflection of the flagellum (Fig. 11). They were stimulated by movements of the bathing medium when the flagellum was not restrained, and by deflection and rotational movements of the flagellum both under saline and also when it was allowed to project into air. Coating the flagellum
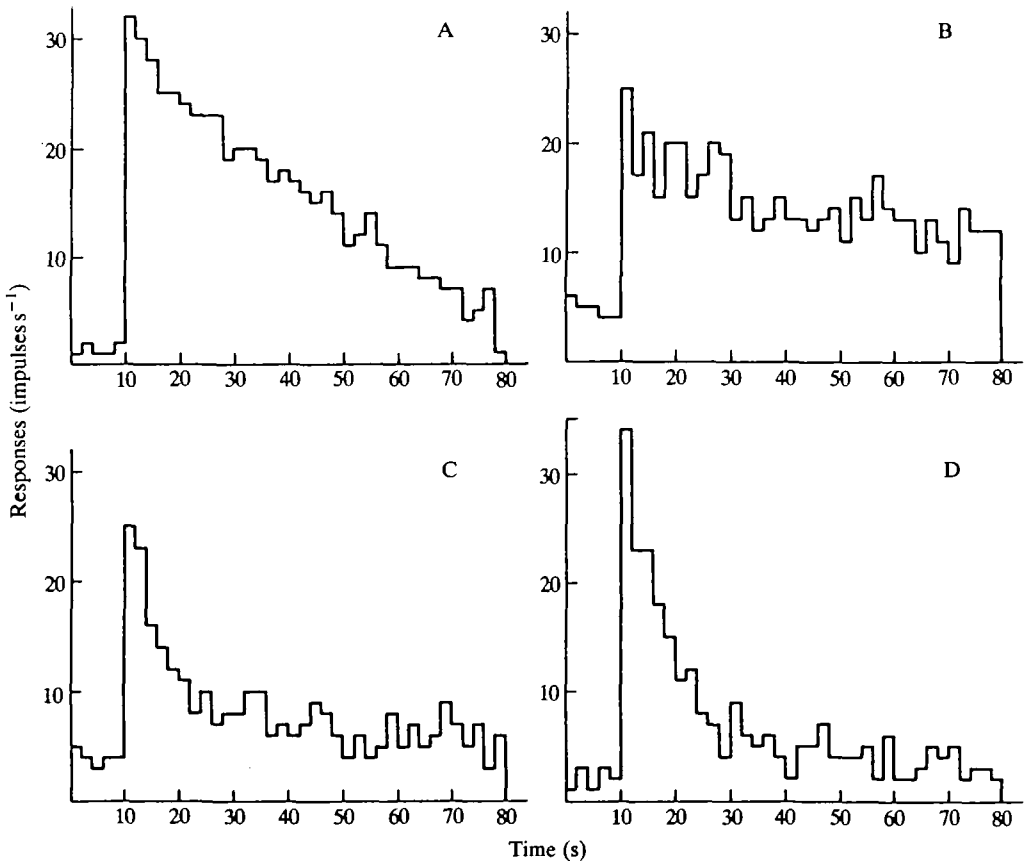

Fig. 11. (A–D) Responses of slowly adapting receptors to a maintained bend of the flagellum. The stimulus was applied at the 10 s mark. The histograms show the frequency of the receptor discharge against time for four different units with different adaptation times. Although not shown here, all adapted within 2–3 min.
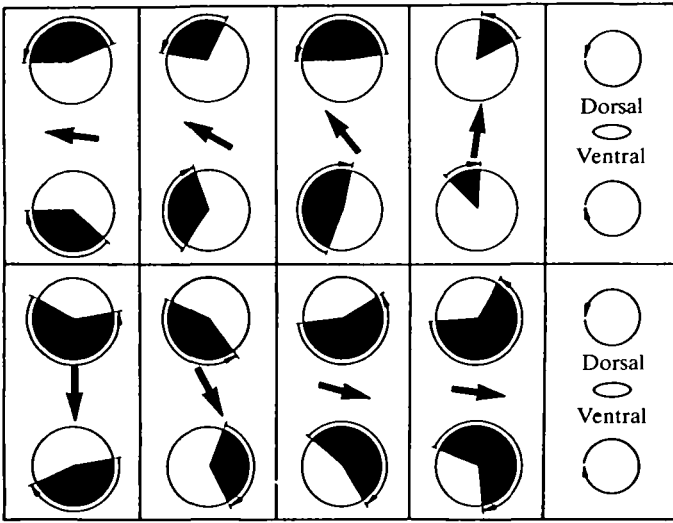
Fig. 12. The responses of eight slowly adapting flagellar receptors to rotation of the flagellum first anticlockwise and then clockwise. The direction of the rotation and orientation of the flagellum are shown at the right of the figure. The receptors discharge when the flagellum is rotated in a particular direction (shown by the arrows) and during part of the cycle (shown by the shaded part in each circle). The large arrows show the mean directional sensitivity of each unit. Some units discharge over a greater part of the cycle than others because the receptors were monitoring joints at different points between the clamp and the eccentric plate. The more proximal segments bend less than distal segments during rotation of the flagellum.

with a non-toxic cosmetic face mask that dried to form a flexible cover (Yves Rocher), did not affect the responses of these units to bending the flagellum. Allowing the outer surface of the flagellum to dry out impaired the flexibility of the arthrodial joints and in such cases slowly adapting units responded less vigorously. They recovered their sensitivity when the joint was remoistened. Plucking all the hairs off the joints containing slowly adapting units did not abolish or alter their responses.

Rotation of the flagellum showed that individual units were specifically sensitive to deflection along particular radii and that, collectively, all directions of flagellar bending are monitored by these receptors (Fig. 12).

The anatomical identity of the slowly adapting receptors has not been established. Such responses could come either from campaniform sensilla or from chordotonal-like internal receptors within the flagellum. Campaniform sensilla have been found on the antennules of *Homarus gammarus, Nephrops norvegicus* and *Crangon vulgaris* (Laverack, 1976) and at the base of the antennae of *Palinurus vulgaris* (Vedel, 1985). These are typically domed structures of about 40 μm in diameter, curved inwards at the apex around a central pore containing a rounded stub (see Laverack, 1976; Vedel, 1985). The receptors occur in rows or clustered around the bases of mechanoreceptor hairs. The only structure found on

the flagellum of *Cherax* resembling such campaniform sensilla is shown in Fig. 9F. On closer examination, however, it is considered more likely that this well-shaped structure is the empty socket of one of the long smooth hairs (see Fig. 9G,J). Broken hairs are rarely seen in the flagella of freshly moulted animals, but are more common in intermoult animals when empty hair sockets such as that in Fig. 9F appear more frequently.

Four chordotonal receptors at the base of the flagellum of the hermit crab have been described (Taylor, 1967) and although no receptors of this kind have been reported in the more distal sections of antennal flagella, this may be due to the difficulty of distinguishing them from receptors associated with the external hairs.

Methylene blue stains of the receptors contained within the flagellum of *Cherax* reveal the extent of the neural complexity within this structure (Fig. 13). Most of the receptors lie along the medial edge of the flagellum, corresponding with the larger number of smooth hairs found there. A consistent feature of the receptor distribution is the alternation between parallel and diagonally oriented rows of receptors in neighbouring segments of the flagellum (Fig. 13A). Small bipolar cells branch into the space between the segments and do not appear to end near hair bases (Fig. 13B). Receptor cells that do project to the bases of hairs appear alone, paired or in groups of three or more (Fig. 13E) and the three types are often found adjacent to one another (Fig. 13C,D).

The antennal motor system

Anatomy

The neuromuscular organization of the antennae of a number of freshwater and marine crayfish and lobsters has been well described (*Astacus fluviatilis*, Keim, 1914; Schmidt, 1915; North American cambarid and procambarid crayfish, Habig & Taylor, 1982; Tautz & Müller-Tautz, 1983; *Euastacus armatus*, Sandeman & Wilkens, 1983; *Panulirus*, Vedel, 1980; Sigvardt, 1977). Six motoneurones control the two muscles that flex and extend the flagellum of the Australian freshwater crayfish *Euastacus armatus* (Sandeman & Wilkens, 1983). Of these one is a common inhibitor, leaving three excitatory extensor axons and two excitatory flexor axons. Of the three extensor axons, one is phasic, one does not respond to forced antennal movements and one is tonic. Of the flexor axons, one is phasic and one is tonic. Methylene blue vital staining of the antennal muscles of *Cherax destructor* reveals an innervation pattern like that of *Euastacus*.

The consequence of this neuromuscular organization is that extracellular potentials in the muscles of the extensor and flexor muscles can be recognized as being either phasic or tonic neurones that respond to forced antennal movements, thus uniquely identifying them. Intracellular responses from cells penetrated in the brain can then be compared with the simultaneously recorded extracellular responses and, if they match, positively identified.

Physiology

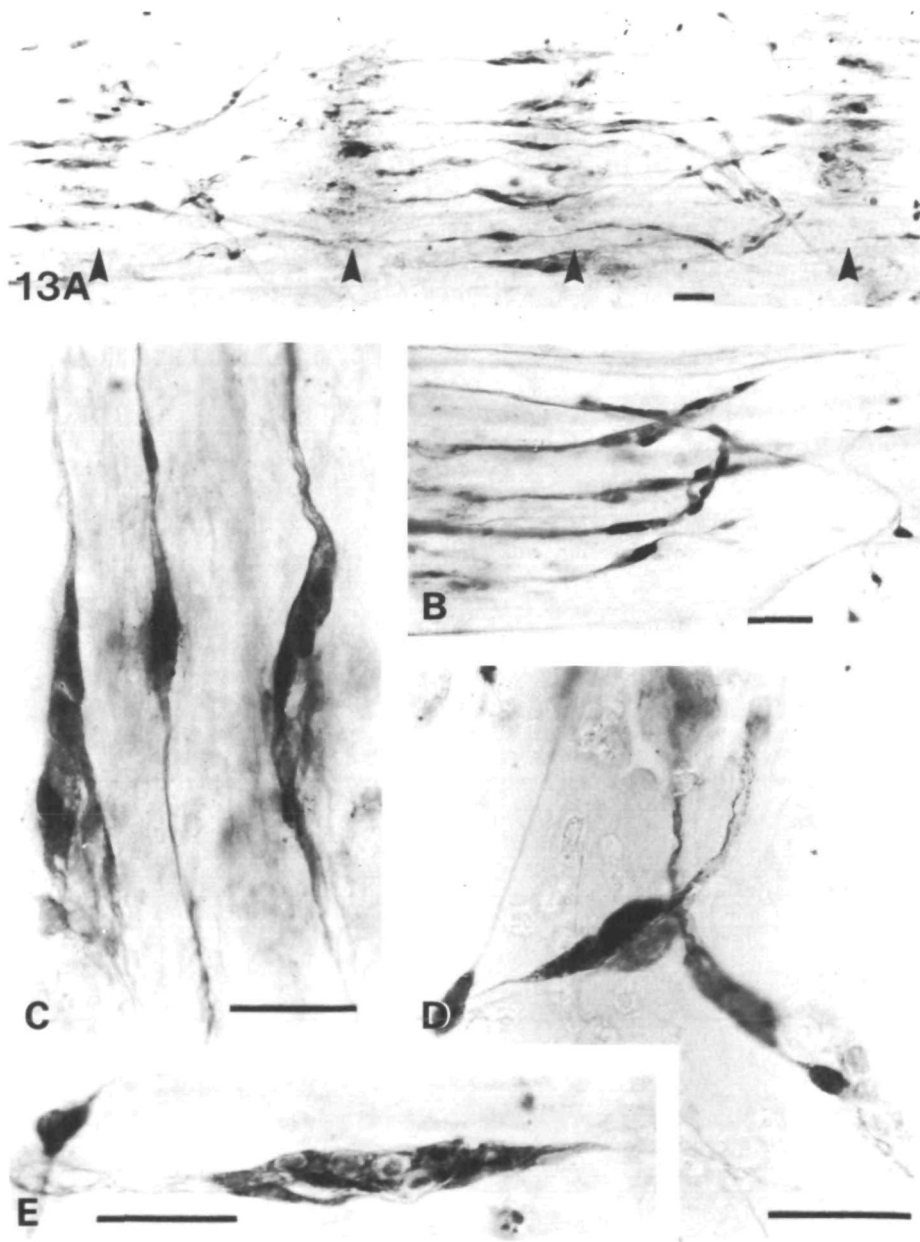Brushing the medial edge of the flagellum resulted in the appearance of

Fig. 13. (A) A strip of tissue removed from inside the medial edge of the flagellum and stained with methylene blue. Pigment cells and the hair bases mark the division between the segments (arrowheads). The orientation of the receptor cells to the long axis of the flagellum alternates from parallel to diagonal in consecutive segments. The base of the flagellum is on the right. (B) A line of small bipolar cells branches in the area behind a row of receptors associated with hairs. (C,D) Receptors associated with the hairs occur singly, in pairs or in larger groups. These three types are often found adjacent to one another in both parallel (C) and diagonally (D) oriented receptors. (E) A multicellular receptor. Calibration bars, 50 μm.

inhibitory potentials in the tonic extensor motoneurone, whereas brushing the lateral edge led to an increase in the frequency of its firing rate. The opposite was true of the tonic flexor. The inhibition and excitation in both cases lasted only as long as the brush continued to move along the flagellum (Fig. 14A).

Flexion and extension of the flagellum about its joint with the carpopodite produced 'resistance reflex'-like changes in the firing rate of the tonic extensor and flexor muscles in that the extensor firing rate increased with flexion and decreased with extension. The reverse was true of the tonic flexors. In these responses the separate effects of movement and the subsequent maintained change of position can be seen. Movement of the joint is the more effective stimulus in altering the firing frequency of the motoneurones (Fig. 14B).

Bending the flagellum was found to have a marked effect on the discharge rate of the tonic extensor and flexor motoneurones. Medial bending resulted in an increase in the discharge rate of the tonic extensor that was maintained while the flagellum was kept in the bent position. Allowing the flagellum to straighten was accompanied by a decrease in the firing rate of the tonic extensor (Fig. 14C). Bending the flagellum laterally was similar to releasing it from a medially bent position and caused a decrease in the tonic extensor discharge; releasing it caused an increase. The opposite was true of the tonic flexor: bending medially resulted in a decrease in its discharge rate, releasing it an increase (Fig. 14C). Lateral bending increased the discharge rate, releasing decreased it. Unitary inhibitory potentials were always present in both motoneurones. Usually of larger amplitude in the tonic extensors, their frequency could be increased by brushing the medial edge of the flagellum. An increase in the frequency of their appearance was also, surprisingly, associated with stimuli that led to an increased firing rate of the motoneurone. However, if the inhibitory potentials are the result of flagellar mechanoreceptor stimulation, as the brushing would indicate, their appearance during antennal deflection and bending could be explained. In both cases the fork used to deflect or bend the flagellum rested against its medial and lateral edges and would therefore have stimulated the mechanoreceptors there.

Discussion

The crayfish *Orconectes* point their antennae at a vibrating probe (Tautz, 1987) and *Cherax* point their antennae at novel visual stimuli. Although the behavioural responses of *Cherax* to vibratory stimuli have not been tested, their very flexible flagella and the sensitive long smooth hairs can be expected to function at least as well in this regard as those of *Orconectes*. The significance of pointing the antennal flagellum directly at a stimulus, whether vibratory or visual, is that an approaching object will be intercepted by the antenna and tactile contact made at the greatest possible distance from the body, i.e. the full length of the antenna. Thus the sensitivity of the antenna to waterborne vibrations may be solely to align the antenna to an approaching object. To be effective the flagellum must remain
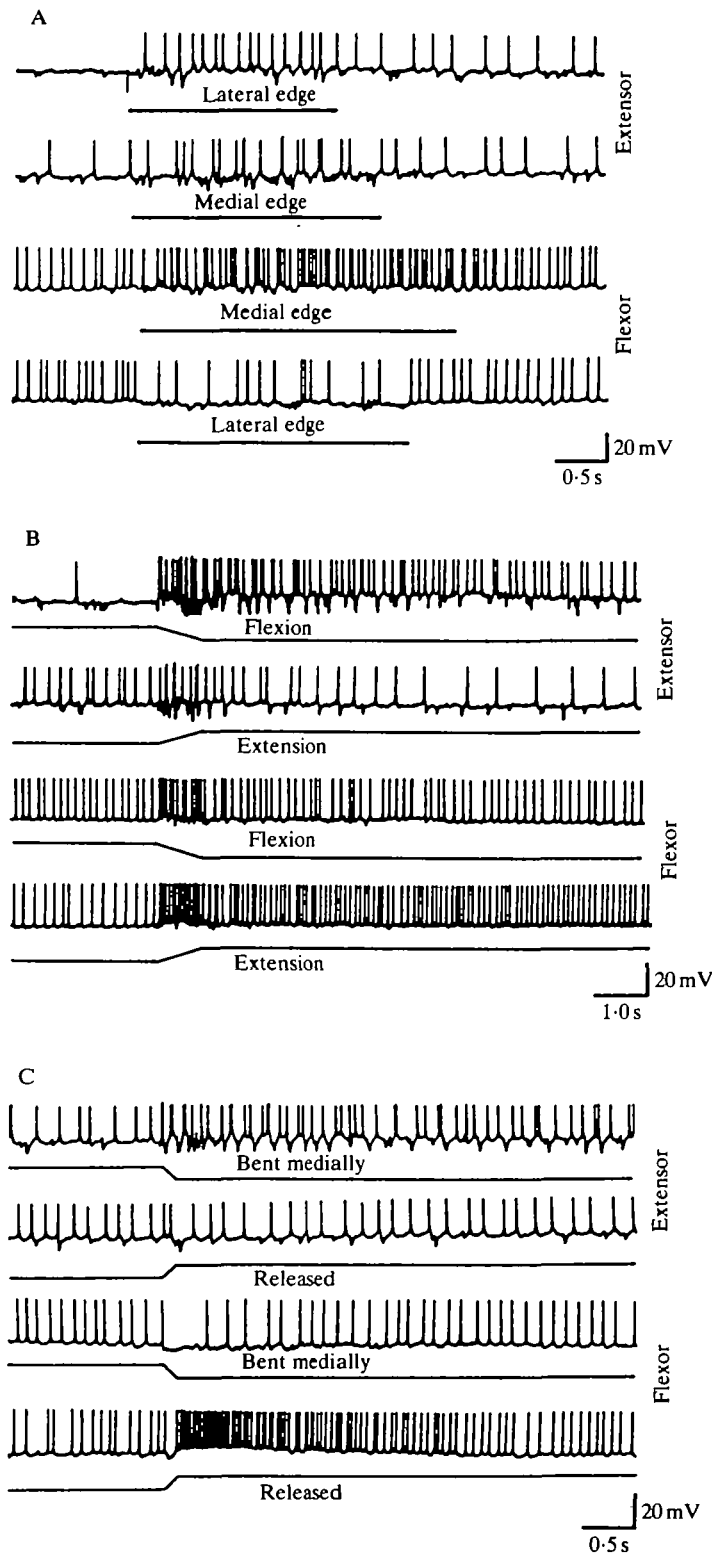
Fig. 14

unbent and in *Cherax* both the inherent mechanical properties of the flagellum and reflexive muscle action contribute to achieve this.

Once contact is made with the approaching object, a different set of receptors is required to extract information from it. This information may include its size, shape, direction and velocity of its movement, chemical nature, surface texture, hardness etc. We do not know which of these the animal can detect with its antennae but an examination of the tactile reflexes, behaviour and receptor characteristics provides us with some indications. Behavioural studies are complicated by their passive or active nature, i.e. stimuli that are applied to the flagella of resting animals, or stimuli that the flagella receive when the animals actively explore their environment. Nevertheless, both provide insights into the role played by the various receptors of the flagellum.

The flagellar receptors and the tactile reflexes

A gentle touch to the medial or lateral edge of the flagellum of seeing or blinded animals results in a small-amplitude withdrawal of the flagellum from the stimulus (Sandeman & Sandeman, 1984). This initial low-amplitude avoidance reflex may be followed by an attack in which the antennal flagellum is drawn rapidly backwards as the animal lunges towards the stimulus (Zeil *et al.* 1985; Sandeman & Varju, 1988). Neither the avoidance reflex nor the following attack are released unless the flagellum is actually touched. Moving a probe near the flagellum is ineffective. Covering the flagellum with nail varnish abolishes both the avoidance reflex and the attack.

The short smooth hairs are insensitive to waterborne vibrations but respond with a sharp phasic burst when they are touched. Their effect on the motoneurones that move the flagellum is of short duration. Receptors on the medial edge of the flagellum excite the flexors. The converse is true of the receptors on the lateral edge. Thus the short smooth hairs are good candidates for the release of the avoidance reflex.

Bending the flagellum laterally or medially affects the activity of the tonic flexor and extensor motoneurones of the flagellum. Bending the flagellum medially excites the tonic extensors. The converse is true if the flagellum is bent laterally. In an intact animal these muscle actions would result in a medially bent flagellum

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Fig. 14. Intracellular responses from tonic extensor and flexor motoneurones to muscles that move the flagellum. (A) Brushing the medial and lateral edge of the flagellum shows that medial edge stimulation inhibits the extensor, lateral edge stimulation excites it. The tonic flexor is excited by medial edge stimulation and inhibited by lateral edge stimulation. (B) Flexion and extension of the joint between the flagellum and the antennal carpopodite produces 'resistance reflex' excitation of the tonic extensor and flexor motoneurones, i.e. flexion excites the extensor, extension excites the flexor. (C) Bending the flagellum medially excites the extensor motoneurones which continue to fire after the movement has stopped. Returning the flagellum to its straightened state results in a decrease in tonic extensor activity and the appearance of inhibitory potentials. The opposite is true for the tonic flexor motoneurone.

being moved forward and straightened, and a laterally bent flagellum being moved backward and straightened. The directionally sensitive, slowly adapting receptors are the most likely candidates for the modulation of these actions.

Forcing the flagellum medially or laterally about its joint with the carpopodite evokes activity in the tonic flexor or tonic extensor motoneurones. This is the familiar resistance reflex known in *Euastacus* (Sandeman & Wilkens, 1983). Painting over the antennal flagellum and thus removing the input from the smooth hairs does not prevent the resistance reflex. The chordotonal organ in the meropodite and carpopodite (Sandeman, 1985) is therefore the most likely sensory input for this reflex.

Active antennal movements

Crayfish have not been seen to explore the contours of a novel object with their flagella in a way that may provide them with information about its shape or size. Instead, their movements in an aquarium seem to be relatively unmodulated by the antennae which are often bent back on themselves while the animals walk along a wall or run into an obstruction.

The rather special mechanical properties of the flagellum may provide a clue to the way in which the organ is being exploited by the animal. The flagellum is tapered from base to tip and the articulating segments enable it to bend close to the point of contact and nowhere else along its length. An asymmetry in the resistance to bending in different directions about its long axis ensures that torsion about the longitudinal axis is minimized. Slowly adapting receptors in the flagellum signal both the extent, direction and location of the bend. Such an organ could be used not only to measure the distance of objects close to the animal but also their mechanical resistance. Both the direction and velocity of motion of an object in contact with the antenna could be detected.

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