MOTOR CONTROL OF MOVEMENTS OF THE ANTENNAL FLAGELLUM IN THE AUSTRALIAN CRAYFISH, *EUASTACUS ARMATUS*

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SUMMARY

1. Voluntary and reflexive antennal movements of an Australian freshwater crayfish are described.

2. An anatomical and physiological study showed that the flagellar flexor muscle is innervated by three motor neurones (a common inhibitor and two exciters) and the flagellar extensor muscle receives four motor neurones (a common inhibitor and three exciters).

3. The discharges of three of the motor neurones to the flexor and extensor muscles (tonic flexor, tonic extensor, common inhibitor) were recorded simultaneously with the movements of the flagellum in intact but restrained animals. Phase relationships between flexors and extensors are not simply antagonistic for all voluntary movements. The common inhibitor is more often in phase with, or leads, tonic extensor activity, than tonic flexor activity.

INTRODUCTION

The movements of animal appendages are the end result of complex central and peripheral processing. In some systems, motor neurone responses are tightly controlled by the sense organs, and the central nervous system influences only the gain of the response, as in many spatial orientation systems (Sandeman, 1981). In other systems, for example the second antennae of crayfish, there is a large 'voluntary' component in which a number of inputs converge centrally, and the resulting motor outputs are a labile mixture of peripheral and central biases.

Some movements of the crayfish flagella are predictable, but in general the appendages are much less influenced by the application of stimuli of a single modality. An interesting additional aspect of the antennae is that their own movements do not have an immediate physical effect on the appendages of other segments. This contrasts with the movements made by a leg which, because it shares the task of supporting the body with the other legs, can be expected to be coordinated with them.

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A body of knowledge about the antennal systems of marine lobsters already exists, and there are some differences between the spiny lobsters, *Palinurus elephas* (*vulgaris*), and the American lobster, *Homarus americanus*. Spiny lobsters have a common inhibitor supplying both the extensor and flexor muscles of the flagellum (Clarac & Vedel, 1975; Vedel, 1980; Rossi & Vedel, 1980), but the American lobster does not (Sigvardt, 1977). Also, the spiny lobsters have a single chordotonal organ which monitors the motions of the last two joints of the antenna; *Homarus* has two.

In this paper we describe the musculature, motor innervation, and proprioceptive sense organs of the antennae of the large freshwater Murray River crayfish. We have also investigated the physiology of the neurones and muscles producing flagellar movements, and the behavioural basis of unrestrained movements of the flagellum.

METHODS AND MATERIALS

Murray River crayfish, *Euastacus armatus*, of medium size (18–22 cm, rostrum to tail), were obtained by trapping in the Murrumbidgee and Tumut Rivers of New South Wales and kept individually in small tanks in the laboratory at temperatures between 16 °C and 18 °C.

Behaviour

To observe and record the antennal movements of active animals, crayfish were suspended with the dactyls of the walking legs resting on a vinyl 'conveyor belt', stretched between two freely turning rollers. The movements of the antennae were monitored with a capacitative sensing device (Sandeman, 1968). Mechanical stimuli were applied to the animal with a probe; electrical stimuli through silver wires inserted through the carapace adjacent to the peripheral sensory nerves. Animals would sometimes walk forwards or backwards on the treadmill for several minutes at a time. Passive leg movements were brought about by moving the belt of the treadmill by hand.

Anatomy

Positions of the muscles and nerve bundles were examined both in fresh tissues stained with methylene blue and in animals previously fixed in Bouin's solution. Axonal projections to the muscles and to the motor neurones in the brain were traced by cobalt staining: cut axons were placed in an isotonic solution of cobalt chloride (0·167 M) at 4 °C for 20–24 h. Filling of the axons was sometimes assisted by applying a current of 0·3 mA. The cobalt was precipitated with ammonium sulphide and the preparations were washed, fixed in formal saline, dehydrated in ethyl alcohol and cleared in methyl salicylate. Whole mounts were photographed or drawn with camera lucida. When back-filling the large nerve bundles of the brain it was found that removing the nerve sheath, and separating the cobalt solution from the brain by two vaseline bridges, prevented extracellular cobalt diffusion and produced far cleaner final preparations.

Physiology

Isolated and perfused brains

The cerebral ganglion was isolated and perfused as described by Ache & Sandeman

980). Basically the method involves removing the anterior 'head portion' of the animal and perfusing the brain and optic lobes through the lateral and medial arteries with cold oxygenated saline.

Isolated antennae

After cutting the antennal nerve close to the brain, the entire antenna was removed from the animal and pinned down in a dish of cold saline. The exoskeleton over the flexor and extensor muscles of the flagellum was then carefully removed and electrical stimuli were applied with suction electrodes to the teased antennal nerve. Synaptic potentials were recorded from the extensor and flexor muscles using 3 M-KCl filled glass micropipettes and the resulting excitatory and/or inhibitory junction potentials were signal averaged by computer (Nicolet, MED-80).

Chronic neural recordings

Animals were mounted in air in a Lucite box using four brass bars with sharpened points fitted into small holes drilled into the branchiostegites (Fig. 1). A footboard beneath the animal, made rough by gluing sand to it, allowed the legs to be passively pushed forwards and backwards. Tactile stimuli to the abdomen and cephalothorax were applied by hand.

Activity from the motor neurones to the flexor and extensor muscles of the flagellum was recorded with flexible polyethylene suction electrodes.

One flagellum was left free to move and its movements were monitored photoelectrically. The other flagellum was immobilized (inset, Fig. 1).

Adjustments of the flexible electrode tips were made until they lay directly over the individual extensor and flexor motor nerves. With the S_3 segment immobilized, these electrodes provided stable records for several hours during movements of the flagellum. Motor activity from the two nerves and movements of the antennal flagellum were recorded on tape and later analysed by computer, using a window discriminator to separate spikes of different amplitude.

RESULTS
Antennal movements

The joints of the basal segments of the antenna allow the flagellum to be moved into any position in space in front of and to the side of the animal. Animals exhibit a wide range of antennal movements but when placed on a treadmill, backward walking was usually accompanied by the antennae being pointed forwards; during forward walking, the antennae pointed backwards. During walking, the antennae are sometimes whipped back and forth in an oscillatory manner.

Imposing leg movements on a resting animal by moving the treadmill produced antennal movements counter to those accompanying equivalent leg movements during walking. For example, while forward *walking* on the treadmill was often accompanied by flexion of the antennae, forcing the treadmill back past the animal invariably produced antennal extension. Similarly, if the legs were suddenly pulled forward by the treadmill, the antennae were flexed.
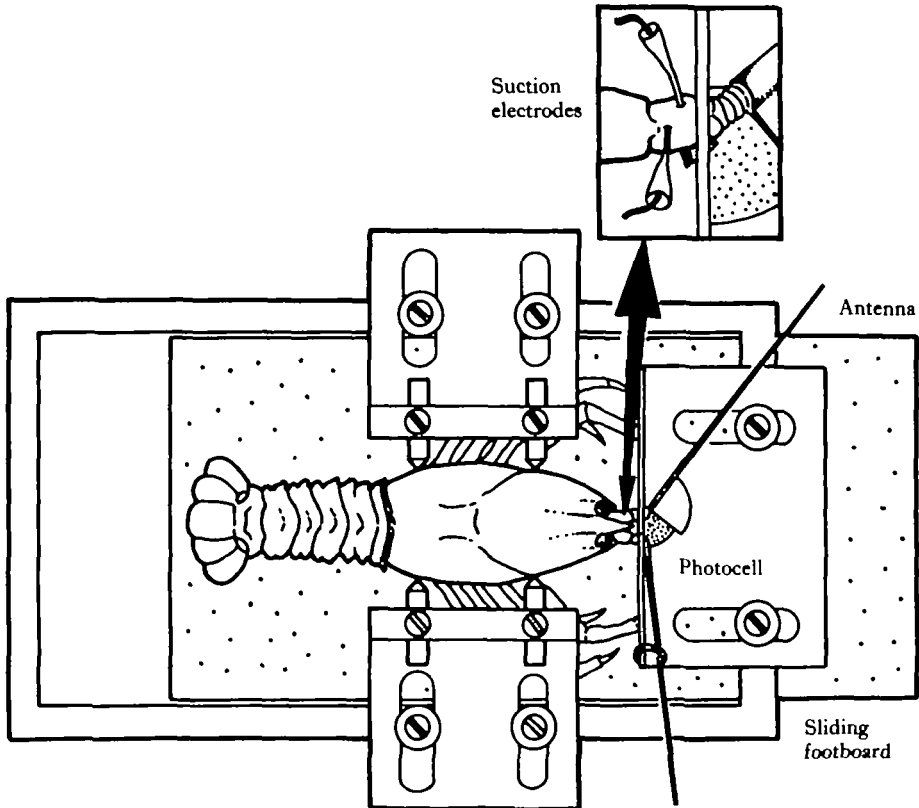
Fig. 1. The method employed to restrain the body and the basal joints of the crayfish antennae, while recording from the motor neurones of the antennal flagellum. The animal is firmly anchored by lateral bars which are fixed to the sides of the box and which grip the heavy carapace. The flagella of both antennae are passed through holes in a stabilizer bar mounted in front of the animal, and the S_3 segment of the antenna is firmly wedged into the hole (inset). One flagellum is tied out to the side, the other carries a piece of aluminium foil which partly shields a photovoltaic cell; any movement of the flagellum is registered as a change in the voltage output of the photocell. Suction electrodes are pushed through small holes in the S_3 segment, and come to lie over the motor nerves (inset). The animal is free to move its legs, abdomen, and the antennal flagellum on the experimental side.

These observations indicate that movements of the legs and antennae are strongly coupled. Further evidence of this association is seen in the response of animals to mechanical stimulation. Stroking the branchiostegite or the leg bases with a blunt object produced an immediate forward extension of the homolateral legs and antenna. In a free-standing crayfish this resulted in the animal tilting towards the stimulated side. If a clamped animal was forcibly tilted to one side, the legs elevated by this movement were extended forward, with the antenna on the same side.

Antennal and leg movements have also been found to be coupled in the escape reflex, with the legs held forwards and the antennae extended, perhaps helping to streamline the animal during backward movement (Wine & Krasne, 1982).

Mechanical stimulation of the anterior areas of the carapace, innervated by the tegumentary nerve, invariably resulted in the protective eye withdrawal reflex. This stimulus could often be used to produce antennal flexion following a branchiostegite-mediated extension. The response was labile, however, and tegumentary input

occasionally produced antennal extension as well. Flexion of the antennae could be more consistently evoked by mechanical stimulation of the uropod or telson, or by pinching the flagellum. A forced extension or flexion of the antennae evoked resistance reflexes, as previously described in the spiny lobster (Vedel, 1980).

The different responses appear to be related to the intensity of the stimulus and to whether the animal is attempting to escape or attack. For example, tapping the animal on the anterior part of its carapace often elicited the defensive posture in which the chelae were raised and the antennae flexed strongly back over the abdomen.

Restricting the movements of all the basal antennal segments by gluing them together and then to the rostrum, had no noticeable effect on the activity of the flagellum, which continued to extend and flex, although through a smaller arc (90°) due to the restriction of the basal joints. Antennal movements in freely moving animals are summarized in Table 1.

Anatomy of the antennal system

Muscles, nerves, and proprioceptor of the terminal segments

The antenna of *Euastacus* is similar in structure to those of the marine lobsters although much smaller than that of the spiny lobster. Like *Homarus*, the endopodite of *Euastacus* has four parts: the ischiopodite, meropodite, carpopodite and flagellum (Sigvardt, 1977). These correspond respectively to segments S_1, S_2, S_3 and the flagellum of *Palinurus* (Vedel, 1980), and we use the latter terminology in this paper (Fig. 2). Within these segments the same joint rotation planes and the same basic system of extensor and flexor muscles are found in *Euastacus* as in *Palinurus*. Muscles arise in S_1 and insert in S_2, and by their contraction produce extension or flexion about the intervening joint. Similarly, muscles arising in S_2 insert in S_3 and move

Table 1. *Summary of antennal movements*

| Activity	Flex	Extend	Flex and Extend
1. Forward walking	B		B
2. Backward walking		B	B
3. Legs forced backwards		B	B
4. Legs forced forwards	B		B
5. Mechanical stimulus to branchiostegite		H	
6. Electrical stimulus to branchiostegite nerve		H	
7. Mechanical stimulus to leg base		H	
8. Lateral compression of cephalothorax		B	
9. Escape response (abdominal flip)		B	
10. Mechanical stimulus to rostrum	H		H
11. Mechanical stimulus to eyes	H		H
12. Sudden visual stimulus		B/H	B
13. Mechanical stimulus to flagellum	H		
14. Forced flexion of flagellum		H	
15. Forced extension of antenna	H		
16. Mechanical stimulus to uropod/telson	B		
17. Rotation of animal – head up		B	
18. Rotation of animal – head down	B		
19. Mechanical stimulus (vigorous) to chelipeds	B		

B = bilateral; H = homolateral.
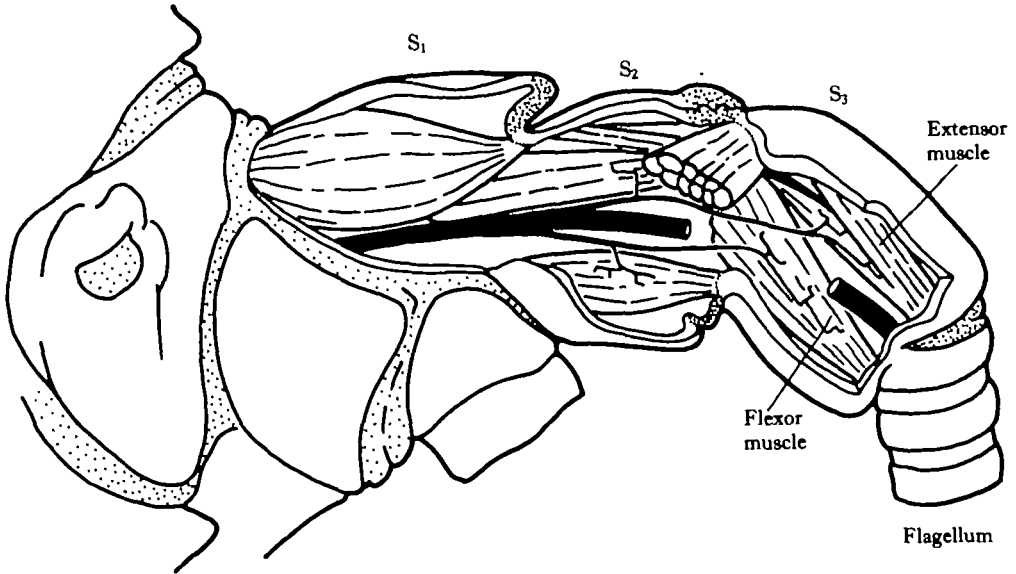
Fig. 2. Drawing of the left antenna of *Euastacus*, seen ventrally, to show segments, and the positions of the extensor and flexor muscles and nerve bundles. Notice that the nerve branches innervating the extensor and flexor muscles of the flagellum separate from the main nerve bundle in S_2.
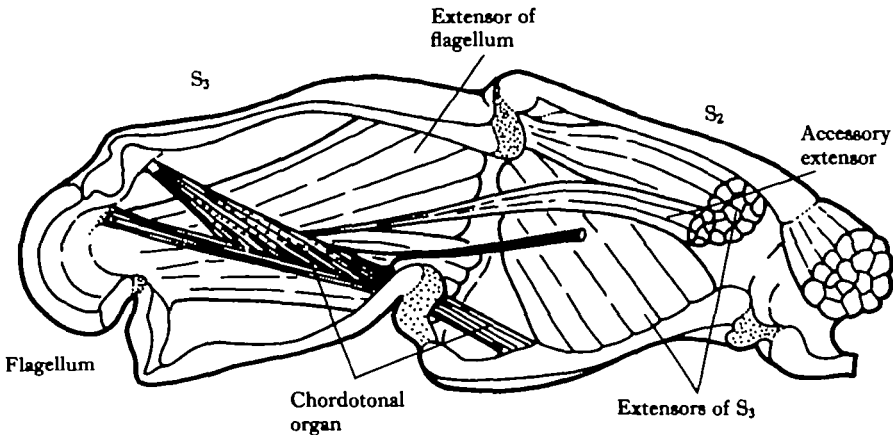

Fig. 3. Dissection of the medial half (saggital view) of the S_2 and S_3 segments of the left antenna to show the position of the chordotonal organ, the extensor, and accessory extensor muscles, of the flagellum.

S_3 on S_2, and the muscles arising in S_3 move the flagellum (Fig. 2). A small accessory extensor muscle arises in S_2 and inserts in S_3 on the apodeme of the flagellum extensor muscle (Fig. 3).

The antennal nerve leaves the brain and divides into several bundles (see below). The main nerve branch penetrates S_1 and then subdivides into a number of smaller branches between S_1 and S_2. The largest of these extends into the flagellum without branching and presumably contains purely sensory axons (Fig. 2). Four other branches can be found. One of these can be followed to the chordotonal organ wh

...ke in *Palinurus*, is situated mainly in S_3 and monitors the movements of both S_3 and the flagellum (Fig. 3). A second, smaller branch (not shown in Fig. 2) innervates mechanoreceptor hairs on the surface of S_3. The two remaining branches supply the flexor and extensor muscles in S_2 and S_3. As these are the muscles producing the antennal movements of interest to us, we have studied their innervation in more detail.

Cobalt-staining of the antennal nerve toward the periphery reveals that eight motor fibres run together in a single bundle in S_2 alongside the main sensory nerve. Halfway along S_2 the motor nerve bifurcates into separate extensor and flexor nerve bundles (Fig. 4). The extensor nerve crosses over the large sensory nerve and contains five axons whereas the flexor nerve contains four. A small fibre (the common inhibitor, see following section), from the original bundle of eight neurones in S_2 bifurcates and sends an axon down both flexor and extensor nerve branches. Each of these branches subdivides to supply the respective muscles of both S_2 and S_3. Two excitatory neurones, in addition to the common inhibitor, are shared by the extensor muscles of S_2 and S_3. One excitatory axon is specific for the S_2 extensor muscle and one for the S_3 extensor muscle.

The flexor muscles of S_2 and S_3 *share* two motor neurones, one of which is the common inhibitor, the other an excitatory axon (Fig. 4). The innervation pattern for the two distal antennal segments in *Euastacus* is therefore the same as that described for *Palinurus* with one exception: in *Palinurus* the S_2 and S_3 extensor muscles have only one common excitatory axon (Vedel, 1980).

An important feature of *Euastacus* which we were able to exploit in the physiological recordings (see below) is that the motor neurones controlling flexion and extension of the flagellum run in completely separate bundles distal to the S_2 segment.

The cobalt studies clearly show that side branches of the motor axons innervating the muscles in S_2 and S_3 are narrower in diameter than the axons continuing to more
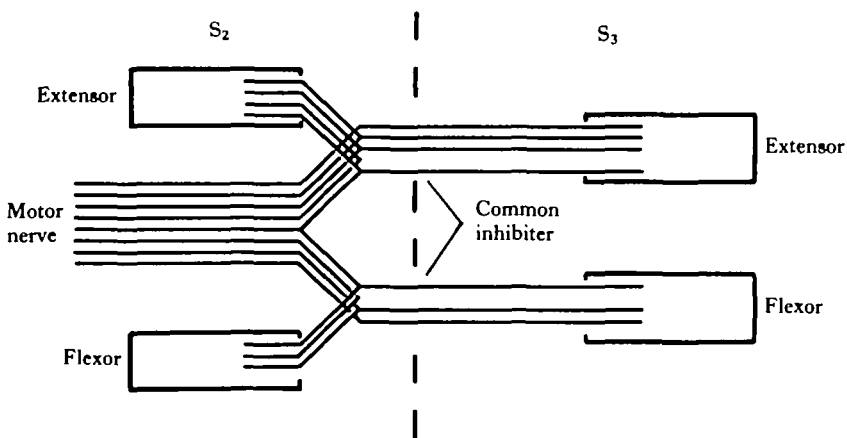

Fig. 4. Schematic diagram of the motor axons to the extensor and flexor muscles of the antenna segments S_2 and S_3 as revealed by cobalt staining. There are no muscles beyond the S_3 segment. Each of the four muscles receives a branch from the common inhibitor. Each muscle also has one excitatory axon specific to itself. Two excitatory axons are common to the extensors of S_2 and S_3, and one excitatory axon is common to the flexors of S_2 and S_3.

A   DORSAL

Optic nerve

Oculo
motor
nerve

Tegumentary
nerve

Antenna
nerve 1

Antenna
nerve 2

Antenna
nerve 3

Oesophageal
connectives

Antenna
nerve 4

Antenna
nerve 3

B   VENTRAL

Median
eyestalk nerves

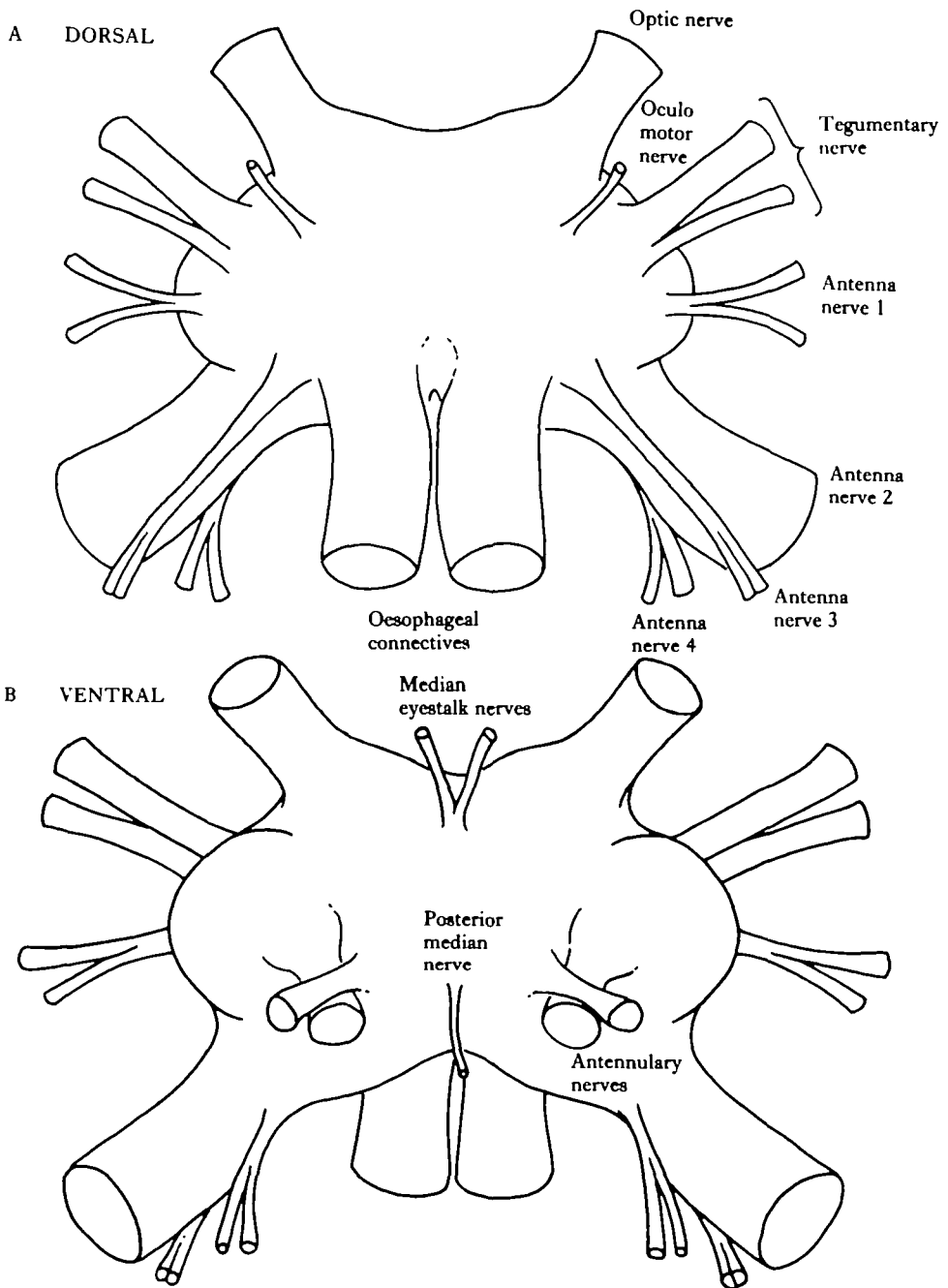Posterior
median
nerve

Antennulary
nerves

Fig. 5. The brain and nerve branches of *Euastacus* seen from above (A) and from below (B).

distal regions. We suppose that this would slow the conduction velocity of action potentials in the side branches, and may result in a nearly simultaneous contraction of the commonly innervated muscles in S_2 and S_3.

Central projections of the antennal motor neurones

The organization of the brain of *Euastacus* is very similar to that of *Cherax destructor* (Sandeman & Luff, 1973). Viewed dorsally, the oesophageal connectives and the optic, oculomotor, tegumentary and antennal nerves are visible (Fig. 5A). Ventrally, the antennular, and the posterior median and anterior eyestalk nerves, can also be seen (Fig. 5B).

The antennal nerve is the largest of all the brain nerves with the exception of the oesophageal connectives. Four main branches can be identified and these divide soon after leaving the brain. The smaller branches (numbers 1, 3 and 4, Fig. 6) innervate the muscles and sense organs of the more proximal segments of the antenna. The motor axons to the flexor and extensor muscles of segments S_2 and S_3, in addition to the sensory fibres from the flagellum, and chordotonal organ are contained in branch 2.

The antennal nerves are similar to those described for another crayfish, *Procambarus clarkii*, (Habig & Taylor, 1982*a,b*). In *Euastacus*, nerves 1, 3 and 4 are equivalent to the antennal motor nerves 1, 4 and 5 respectively of *Procambarus*. Nerve 2 is a single root in *Euastacus* and is equivalent to the combined motor nerves 2 and 3, and the antennal nerve, of *Procambarus*.

Back-filling the four antennal nerve branches of *Euastacus* with cobalt shows that, with one exception, all the motor axons filled have their cell somata distributed in two lateral groups on the dorsal side of the brain (Fig. 6A, B, C, D). The somata of the motor neurones in branch 2, which include the motor fibres in segments S_2 and S_3, are among the largest in the cluster. One axon in this branch has its cell soma located medially and some distance from the others (Fig. 6B).

Physiology of the antennal system

Central effects on the motor neurones

The antennal motor neurones in a semi-isolated, perfused head of *Euastacus* will continue to respond reflexly to forced movements of the flagellum for several hours. These resistance reflexes, which are similar to those described in both *Homarus* and *Palinurus* (Sigvardt, 1977; Vedel, 1980), are generated by sensory feedback from the chordotonal organ in S_2/S_3. In the isolated preparation, reflex activity provides a means of producing activity in selected motor neurones against which the modulating effects of other inputs to the brain can be tested. Our procedure was to flex and extend the flagellum with an electromagnetic device while recording the activity from the motor neurones in S_3 with suction electrodes prior to and during electrical stimulation of fibre tracts in the roots and connectives of the brain.

Depending on the site of stimulation (again using a suction electrode), reflex discharges of the motor neurones were either enhanced, inhibited, or unaffected by driving other inputs to the brain (Fig. 7). Ipsilateral inputs predominate in modulating the reflex response, with the effective contralateral inputs being limited to fibres in the optic nerve and the oesophageal connectives, both of which contain interneurones.

Innervation of the flexor and extensor muscles of the flagellum

During stimulation of the antennal nerve, in an isolated antenna, intracellular recordings were made from the flexor and extensor muscles to determine innervation
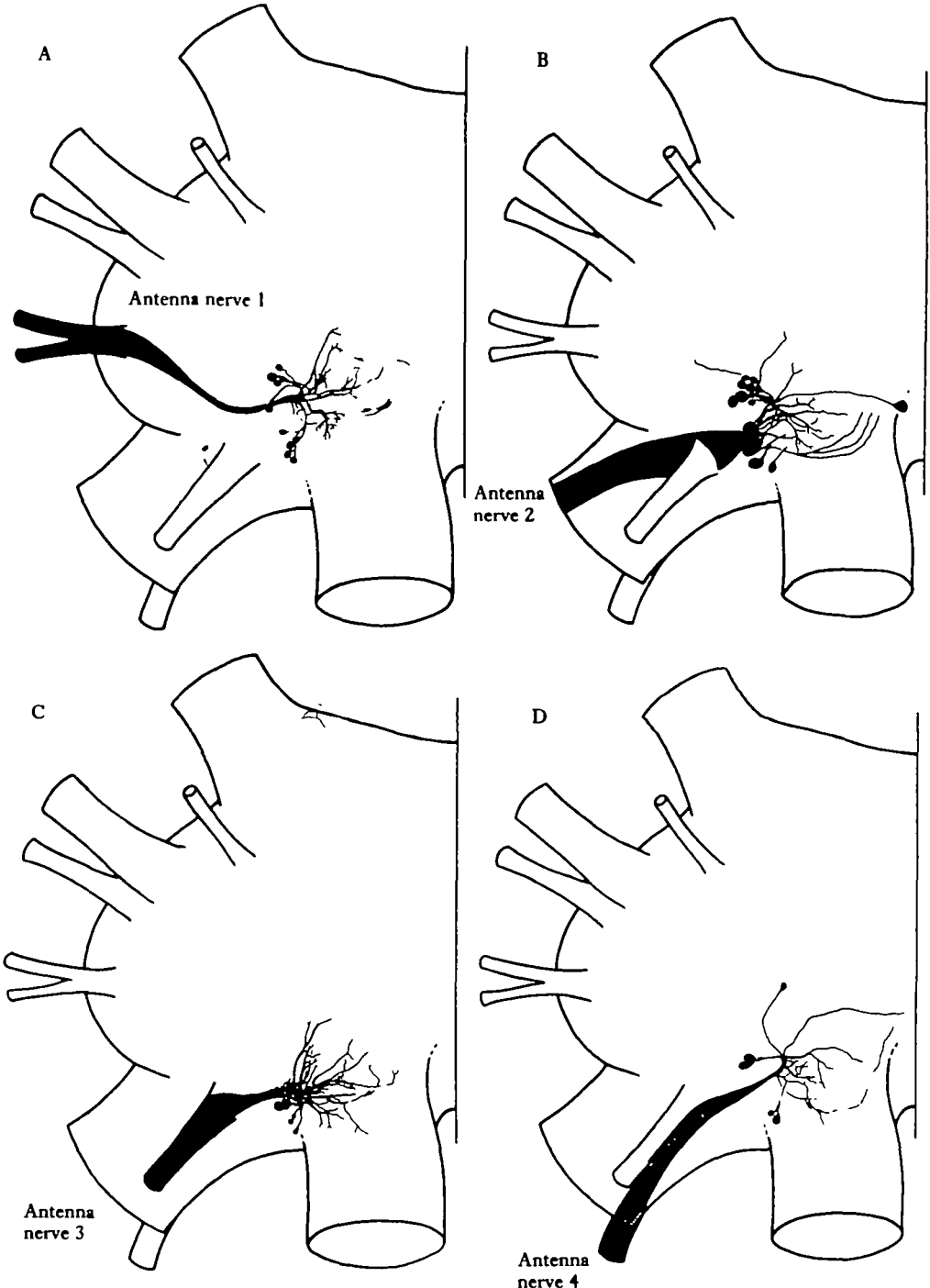
Fig. 6. A–D. The positions of the cell somata, and central arborizations of the antennal motor neurones, as revealed by back-filling the four branches of the antennal nerve with cobalt chloride. Motor neurones controlling the muscles in S_2 and S_3 are among those stained in branch 2.
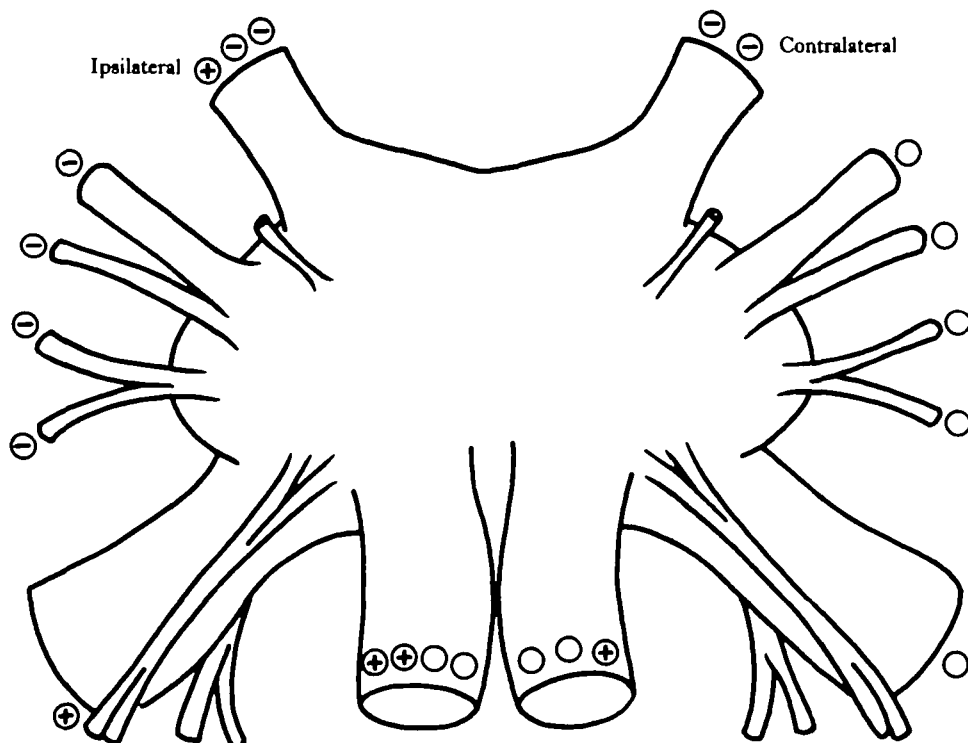
Fig. 7. Enhancement (+) or inhibition (−) of the antennal resistance reflexes can be produced by applying electrical stimulus trains to bundles of axons located in the nerves shown in this figure. An empty circle means that stimulation had no observable effect. The strongest enhancement of the reflex was evoked by stimuli applied to the ipsilateral connectives, near the giant axons.

patterns. Single stimulus pulses of graded intensity steps were applied, resulting in junction potentials of varying amplitudes in the muscles, which were averaged over 20 sweeps. The number of axons innervating individual muscle cells was determined by observing the all-or-none increments in junction potential amplitude associated with increases in stimulus intensity.

Flexor muscle cells responded either with a single amplitude excitatory junction potential, or one which increased in size by one increment at higher stimulus intensities. Still higher stimulus intensities frequently resulted in a decrease in the size of the junction potentials. This effect was reversible and we interpret it to be caused by an inhibitor axon (Fig. 8). Flexor muscle cells are therefore innervated by up to three axons, one of which is an inhibitor.

The cobalt fills of the antennal nerve showed one axon with branches to both the extensor and flexor muscles (Fig. 4). To investigate whether this was the common inhibitor, like that found in *Palinurus*, we stimulated the peripheral branch of the nerve leading to the extensor muscle while recording intracellularly from the flexor muscle. In an isolated antenna, the only action potential which could possibly reflect to the flexor muscle would be that of the branched axon. When this experiment was done, we found that hyperpolarizing junction potentials could be recorded from some of the flexor muscle cells. These inhibitory potentials appeared after a long latency (about 10 ms), had a long duration, and showed a pronounced facilitation with

repetitive stimulation (Fig. 9). In fact, no response was observed with single pulse stimulation (top trace).

In summary, the extensor and flexor muscles of the antennal flagellum are inner-vated by five and four motor fibres respectively, each including a common inhibitory axon. This pattern of innervation is the same as that described for *Palinurus* (Vedel, 1980), a spiny lobster, whereas the more closely related lobster *Homarus*, lacks the common inhibitory cell (Sigvardt, 1977).

Behavioural physiology

In these experiments, motor output to the muscles of the flagellum (S_3 flexor and extensor) was recorded simultaneously with the movements of this appendage. An
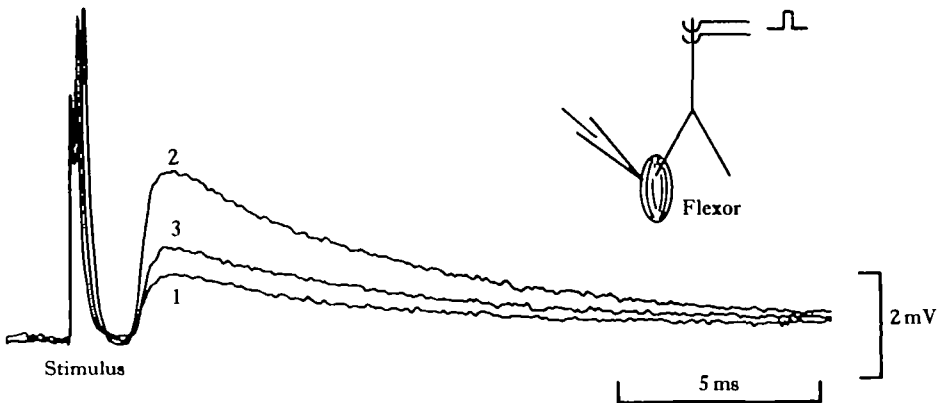

Fig. 8. Intracellular recordings from the flexor muscle of the flagellum in an isolated antenna. Stimuli were applied to the flexor nerve bundle, and a microelectrode was placed in the exposed muscle (inset). A gradual increase in the intensity of the stimulus produces two changes in the lowest threshold muscle potential (trace 1). Trace 2 represents an intermediate stimulus intensity while trace 3 is in response to the strongest stimulus.
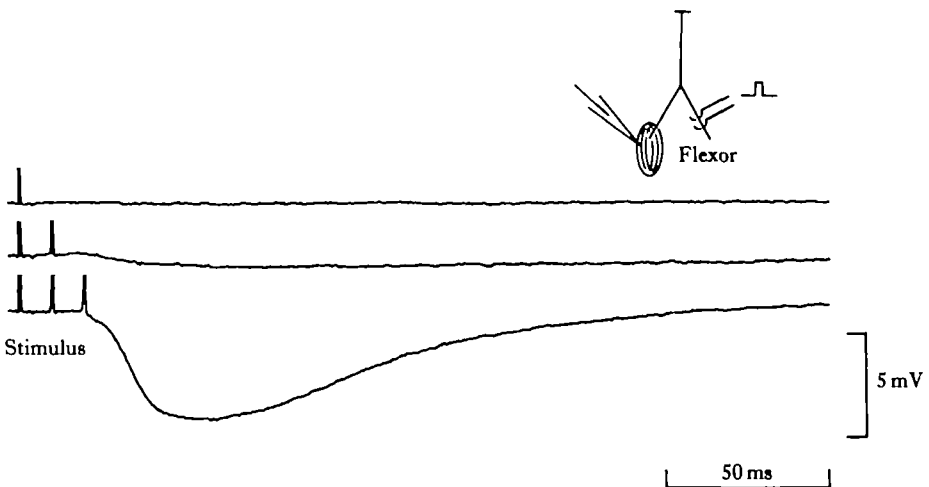

Fig. 9. Intracellular recordings from the flexor muscle of the flagellum in an isolated antenna. Stimuli were applied to the extensor nerve bundle, and a microelectrode was placed in the exposed flexor muscle (inset). Repetitive stimulation (lower trace) produces a long lasting hyperpolarization in the muscle cell.

analysis of this type of data is the most direct approach to gaining insights into the organization of the motor system, including its central circuitry and proprioceptive feedback. The antennal flagellum is ideally suited to these experiments since it moves about a 90° arc in a single plane, (a movement easily recorded photoelectrically), and its flexor and extensor motor nerves are accessible.

Four motor neurones could be positively identified in all *en passant* recordings. The tonic exciters to the flexor and extensor muscles were generally spontaneously active and both discharged reflexly to forced extensions or flexions, respectively, of the flagellum. The common inhibitor often fired spontaneously as well, and its spikes (usually small) appeared simultaneously in both nerve recordings. The spike of the phasic exciter to the S_3 extensor muscle was also easily recognizable as the largest spike in the extensor nerve – it was recruited only when rapid antennal movements occurred. The third excitatory motor neurone to the extensor muscle was more diffi-cult to distinguish because its spike amplitude was similar to that of the tonic exciter. However, this neurone, which also innervates the S_2 extensor, was not active during imposed resistance reflexes and could therefore be separated from the tonic and phasic exciters.

We have concentrated on the activity of the tonic extensor and flexor motor neurones, and on the common inhibitor, since these neurones were always active during flagellar movements. The relatively short 'episodes' used to illustrate motor activity have been taken from many hours of continuous recordings. Motor neurone activity is represented as instantaneous frequency in the following histograms together with the analogue signal of the corresponding flagellar movements.

Imposed movements

Forced extensions and flexions of the flagellum produced the expected bursts of activity for a resistance reflex in the tonic exciters, i.e. extensor activity follows flexion and flexor activity follows extension.

The apparent simplicity of the resistance reflex demonstrated by such experiments is nevertheless misleading. It is known, for example, that reflex responses of the motor
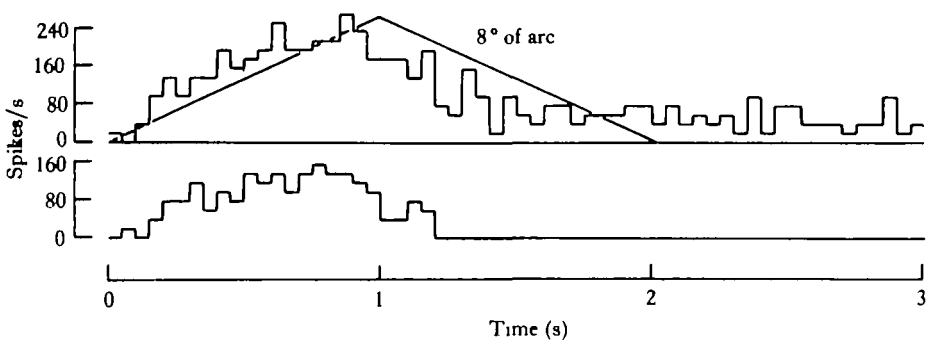

Fig. 10. Enhancement of the resistance reflex by mechanical stimulation of the ipsilateral branchio-stegite. The responses of the tonic extensor motor neurone are plotted as a frequency histogram. The flagellum was flexed and then extended through 8° of arc. The histogram traces show the resistance reflex without (lower), and with (upper), simultaneous stimulation of the branchiostegite. The stimulus is shown in the top trace.

neurones in *Palinurus* are significantly affected by the behavioural state of the animal (Vedel & Clarac, 1975), and our own experiments in which we stimulated the brain roots in *Euastacus* (Fig. 7) indicate that the gain of the resistance reflex can be controlled by sensory inputs other than those of the flagellum. Similarly, mechanical stimulation of the branchiostegite in the intact animal, applied during forced movements of the flagellum, resulted in a more vigorous response in the extensor motor neurone, presumably reflecting a summation of excitatory influences from two sources (Fig. 10; see also Table 1).

During active, non-reflexive movements it is difficult to interpret how much, if any, of the activity in the motor neurones is generated by the resistance reflex. In some cases, for example, we could demonstrate suppression of reflex activity in the intact animal by experimental manipulation of the antenna in conjunction with voluntary behaviour (Fig. 11). These results suggest that input to the brain from the chordotonal organ can be overridden or otherwise centrally controlled since there is no associated muscle capable of resetting the tension on this organ peripherally, thereby
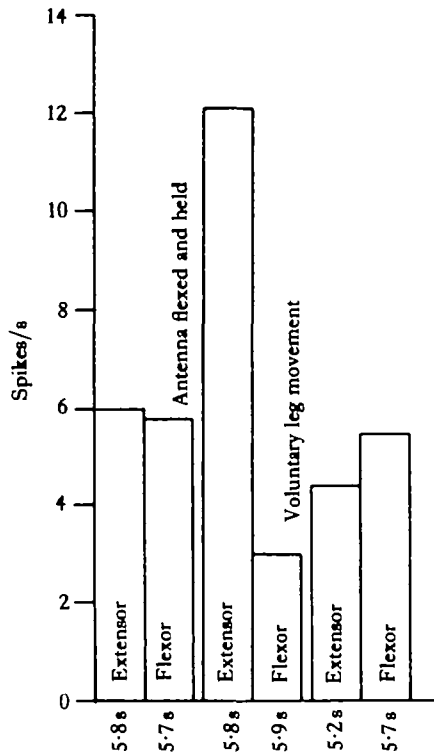

Fig. 11. Changes in resistance reflex discharges after voluntary movement. The first pair of columns show the relative discharge frequencies in the tonic extensor and flexor motor neurones with the flagellum resting in an extended position. The flagellum was then flexed and held in this position, resulting in increased extensor, and decreased flexor, activity (second pair of columns). Subsequent voluntary leg movements, lasting about 5 s, were followed by a marked change in the relative firing frequencies of the extensor and flexor motor neurones, although the flagellum remained in the flexed position (third pair of columns). The time (in seconds) over which spike activity was averaged for each behaviour is indicated below the columns.
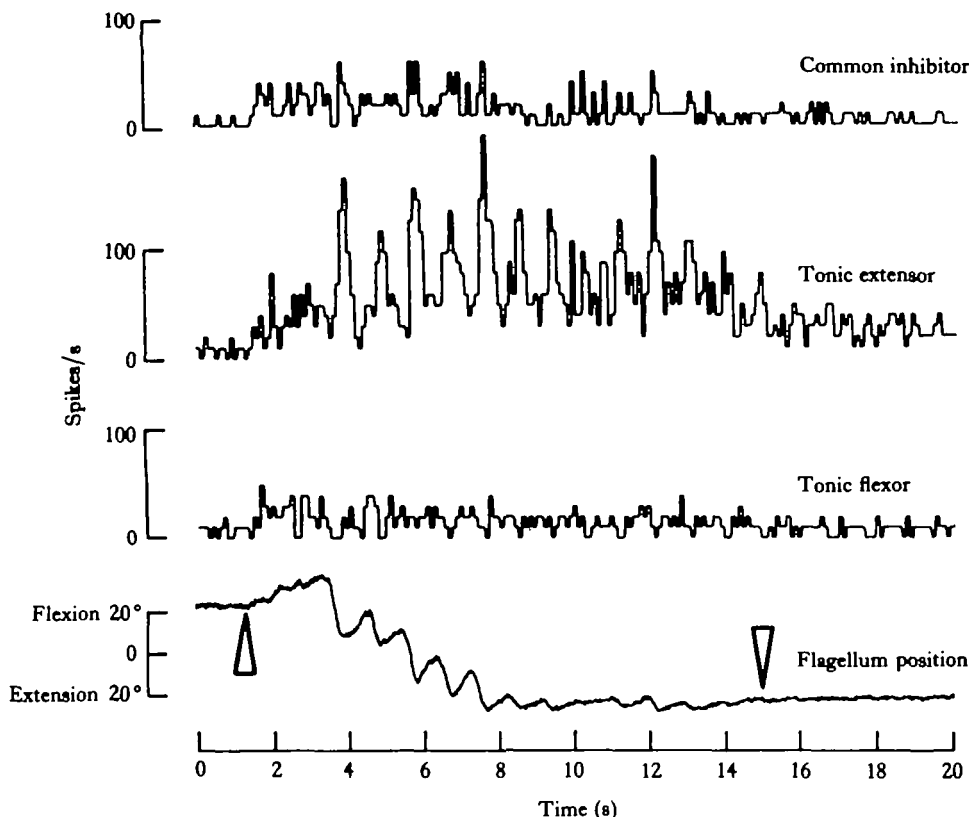
Fig. 12. Antennal movements (lower trace) produced by forcing the legs back and forth with the foot-board. Movements of the legs were made by hand and start and end at the arrows. Responses of the common inhibitor, tonic extensor, and tonic flexor motor neurones are plotted as frequency histograms in this and subsequent figures. The amplitude of flagellar movement, in degrees'of arc, is indicated against the lower trace. 0° corresponds to a flagellar position about 45° to the longitudinal axis of the animal.

modifying the reflex-generated activity. The mechanism of central control remains to be described.

Reflex antennal movements could also be induced by imposing movements on the legs while the animal was clamped in the experimental box (Fig. 1). When we moved the legs of an animal backwards and forwards, by moving the baseboard on which its legs rested, we evoked oscillatory motions of the flagellum that were in antiphase with the movement of the board (Fig. 12). A shift in the position of the flagellum also occurred so that after several oscillations the flagellum came to rest fully extended.

Records of motor neurone activity during voluntary movements did not show the expected degree of correspondence between spike discharge and movement that was evident when the flagellum itself was forced to move (cf. Fig. 10). Here, the flexor motor neurone kept up a more or less constant barrage whereas the tonic extensor fired a series of bursts. The common inhibitor also fired in bursts, many of which accompanied those of the tonic extensor. The resulting flagellar oscillation seemed to be the result of bursts of spikes by the tonic extensors working against a maintained flexor discharge (Fig. 12). In this case, the movements imposed on the legs were relatively low and the phasic exciter neurones were not activated.

Spontaneous movements

The resistance reflex of the antenna is a consistent experimental phenomenon. However, its role during various patterns of behaviour is not understood in any great detail. An example where such a reflex might be involved is when an animal sweeps its antenna against an obstruction. This can be readily tested in *Euastacus* where antennal flexion can be repeatedly evoked by pinching the telson. Recordings were made of the motor neurone discharges, first when the flagellum was allowed to flex unimpeded, and then when it was blocked about half way through its excursion. In its extended position, the tonic extensor was again more active than the flexor and inhibitor neurones (Fig. 13A). Pinching the telson produced a rapid flexion of the flagellum and a burst of activity in all three motor neurones. Following a few additional movements, the flagellum came to rest in a flexed position where the tonic flexor was now more active than the extensor. The inhibitor stopped firing (Fig. 13A).

The events observed in the motor neurones when the flagellum flexed against a stop suggest a positive feedback-driven response in the motor neurones (Fig. 13B). Here, the motor neurones continued to discharge for about 6 s, before the resting discharge characteristic of the flexed flagellum was assumed. The activity of the flexor motor neurone only slightly preceded that of the extensor and often seemed to be synchronous with it and the common inhibitor.

In contrast, the actions of the tonic flexors and extensors took on more antagonistic roles during movements of the flagellum produced by the animal while it explored the baseboard with its legs (Fig. 14). Here, flexions of the flagellum clearly followed activity by the flexor motor neurone while extension followed extensor activity.

The discharge 'profile' of the common inhibitor more nearly resembles that of the extensor than that of the flexor in these recordings. A close examination of the episode in Fig. 14 nevertheless reveals a number of clear exceptions to this generalization. At *a*, a short burst from the inhibitor and extensor are coincident, but at *b* and *c* the discharge of the common inhibitor corresponds more closely with flexor activity. Antennal flexion followed by a rapid extension at *c* is accompanied by an expected flexor and then extensor burst of activity (the inhibitor fires during extension). The rapid flexion at *d* is preceded by a sharp increase in activity in both the inhibitor and extensor, both of which shut off during the flexion but increase again rapidly with the result that flexion of the flagellum is arrested. Bursts of activity in the inhibitor at *e* and *f* are coincident first with extensor activity and then with flexor activity.

Walking animals frequently sweep their flagella back and forth at about 2 Hz. These 'oscillations' were accompanied by motor neurone discharges as shown in Fig. 15. Here flexor and extensor activity were rigidly antagonistic and common inhibitor activity was tightly coupled with the tonic extensor. However, common inhibitor activity, when it occurred, usually preceded the discharge bursts in the exciter motor neurones. To test this point we sampled periods of rest and movement similar to those shown in Fig. 14 and constructed latency histograms for one spike train relative to the others. The discharge of either the common inhibitor, tonic extensor, or tonic flexor motor neurone was used alternately as the trigger pulse while the spike latency for the other neurones was sampled over a period of 25 ms following the trigger spike. The results, taken from 1000 trigger samples in each case, bear out the observation th
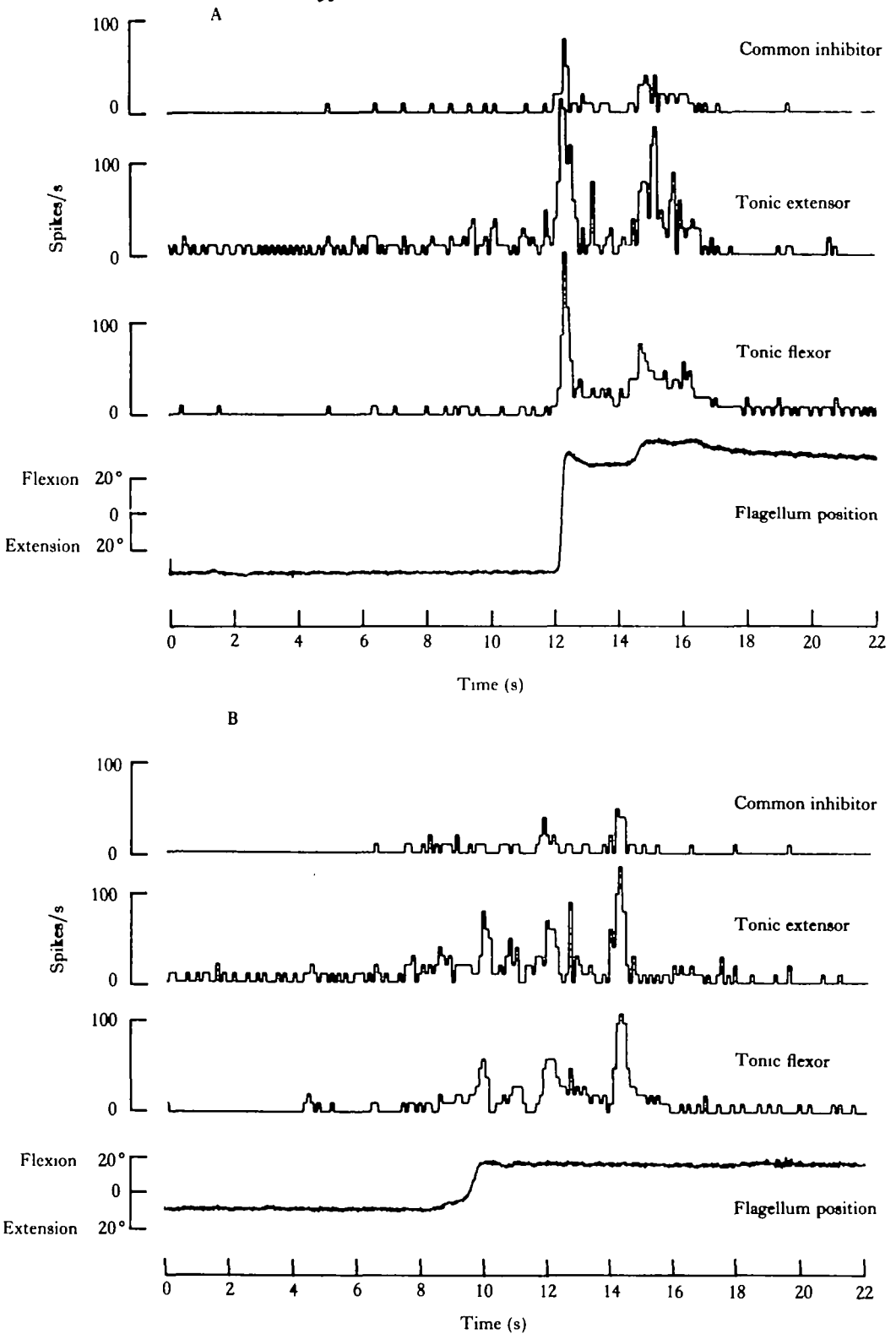
Fig. 13. Responses of antennal motor neurones when the flagellum extends freely (A) or against an obstruction (B).
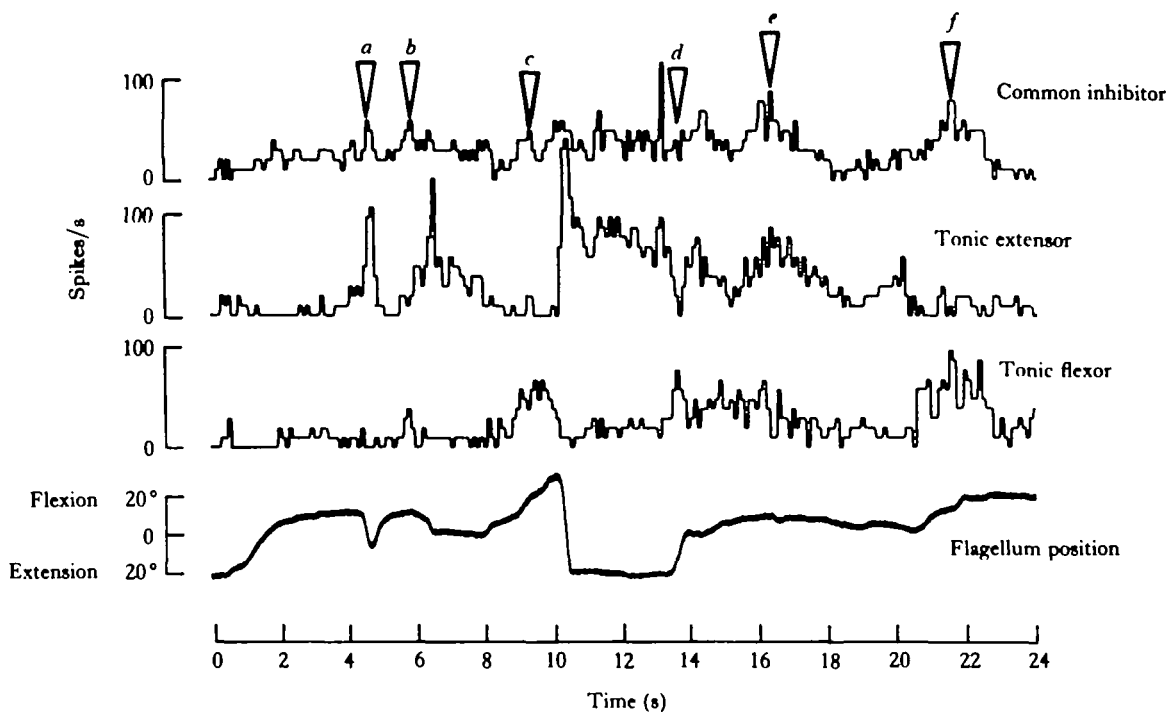
Fig. 14. Responses of antennal motor neurones during voluntary movements of the flagellum (see text for details).
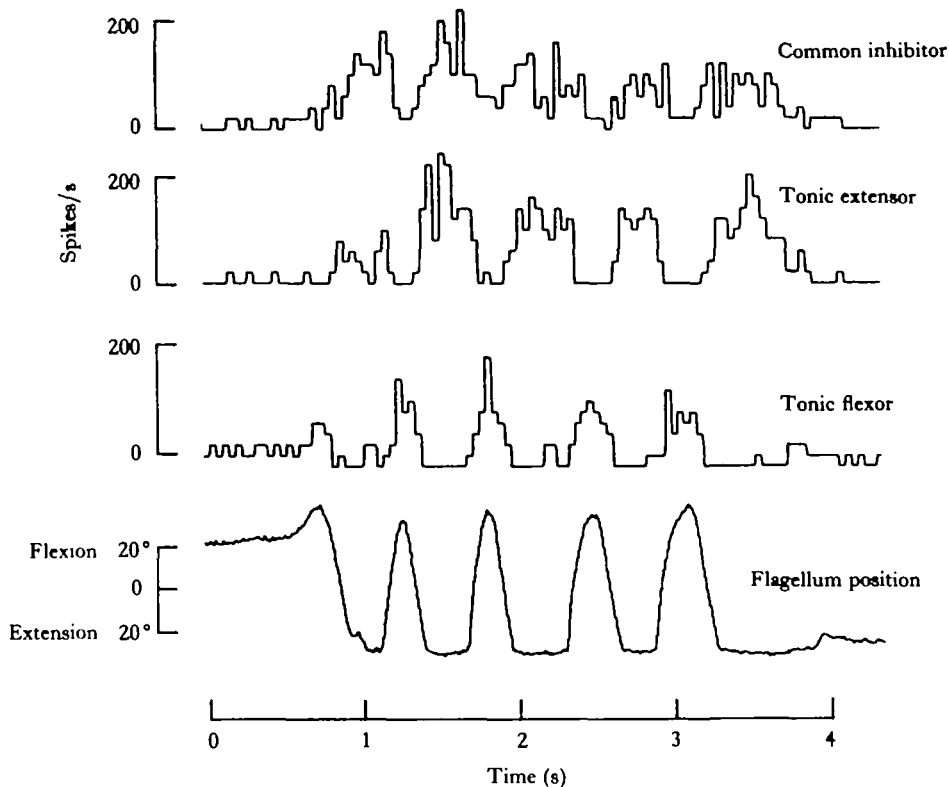

Fig. 15. Responses of antennal motor neurones during voluntary oscillations of the flagellum.
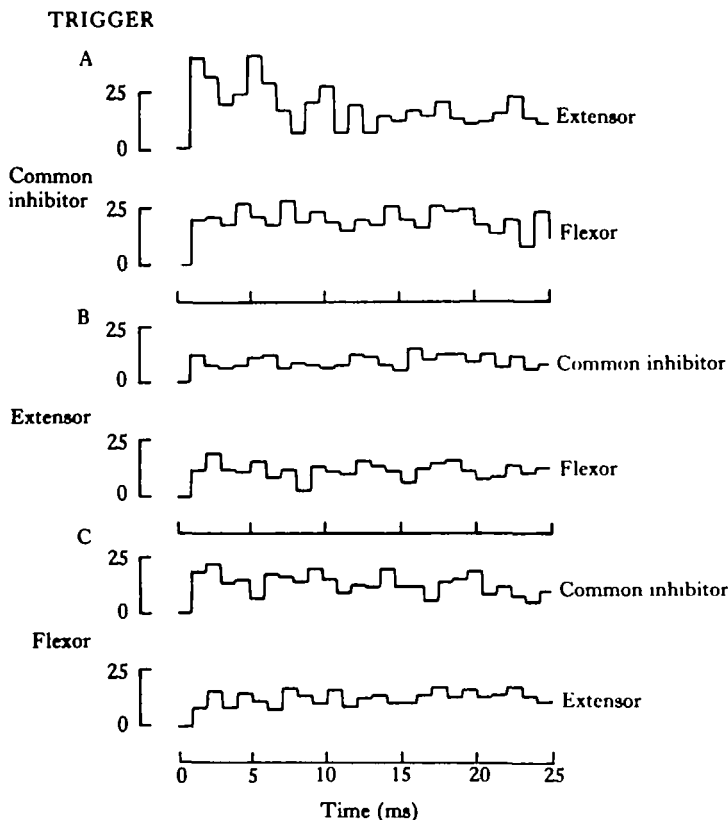
TRIGGER

Fig. 16. Latency histograms showing the phase relationships between three antennal motor neurones. In each pair of histograms, the discharge of one neurone was used to trigger the computer sampling of activity from the other two neurones. The triggering neurone is indicated to the left of each figure.

the common inhibitor more often precedes than follows extensor motor neurone discharges (Fig. 16A). Other relationships between the three motor neurones are less obvious. There is a tendency for extensor activity to be followed more often by the flexor than common inhibitor (Fig. 16B), and for flexor activity to be followed more often by the inhibitor than the extensor (Fig. 16C).

DISCUSSION

Our results show that the anatomy and motor control of the flagellum in *Euastacus* is similar to that of *Palinurus elephas* (*vulgaris*) (Vedel, 1980). The extensor and flexor muscles of the flagellum are similarly arranged, they are (with one exception) supplied with the same number of motor axons and there is a common inhibitor neurone which innervates both of the antagonistic muscles. In *Euastacus*, the chordotonal organ which monitors the movement of the flagellum is found in the same place in the antennae as in *Palinurus*, and the accessory muscle has no obvious connection with the receptor organ. In both animals, the extensors and flexors of S/S_3 also share common exciter motor neurones. In *Euastacus*, this anatomical

feature was reflected behaviourally by the fact that, in most situations, the S_2 and S_3 segments functioned as a single segmental unit.

The similarities in the anatomy of the systems are also reflected in the physiology of their motor activity during voluntary movements: discharges of the common inhibitor in both animals, for example, can accompany either the flexor or the extensor motor neurone (Fig. 14) (Vedel & Clarac, 1975; Vedel, 1980). During rapid voluntary oscillations of the flagellum however, inhibitor and extensor discharges in *Euastacus* are clearly synchronous (Fig. 15).

With regard to the action of the common inhibitor, our results can be taken to lend some weight to the suggestion by Ballantyne & Rathmayer (1981) that the inhibitor prevents residual tension from building up in the tonic muscles during rapid oscillatory movements. Certainly the fast voluntary oscillations of the flagellum in *Euastacus* are accompanied by a high rate of activity in the common inhibitor (Fig. 15). In contrast, slower antennal movements evoked by moving the legs are accompanied by significant extensor activity and much less inhibitor activity and the flagellum indeed gradually extends (Fig. 12).

The same diffuse facilitation of the chordotonal organ-mediated resistance reflex of *Palinurus* (Vedel & Clarac, 1975) was found in *Euastacus*, although with slightly different types of sensory input. Facilitation of reflexly-evoked extensor activity occurs in *Palinurus* when the homolateral legs are forcibly raised, whereas in *Euastacus* it occurs with mechanical stimulation of the branchiostegite. Flexor activity in both animals is enhanced by manipulation of the telson and uropods. The results from stimulation of the brain roots of *Euastacus*, however, suggest that there are many other facilitatory and inhibitory inputs yet to be explored in this system.

Against the similarity of the antennal systems in *Euastacus* and *Palinurus*, the patently different organization of the flagellar system in the American lobster (Sigvardt, 1977) takes on greater significance. The absence of the common inhibitor in *Homarus* has no obvious bearing on the differences in antennal behaviour (Vedel, 1980), in fact, *Euastacus* is much more like *Homarus* in that it does not use its antennae for defence. *Homarus* and *Euastacus* are also phylogenetically more closely related, belonging to the same infraorder, the Astacidea, while *Palinurus* belongs to the Palinura (McLaughlin, 1980).

In *Euastacus* and *Palinurus* control of the flagellar motor neurones by the chordotonal organs probably represents the lowest level in the system. Motor neurone discharge rates in both animals reflect to some extent the resting position of the flagellum, and tonic, position-specific receptor cells in the chordotonal organs, which could be directly responsible for this, have been found in *Palinurus* (Rossi & Vedel, 1980). The flagellar resistance reflexes are similar to those appearing at many joints in other appendages and, like these, this level of appendage control is overlayed by the integration of the inputs from a number of parallel systems (Evoy & Ayers, 1982). It is in the investigation of the integration of these parallel inputs that the real interest and challenge lies. Unlike the cyclically repeated leg movements, or tightly stimulus-coupled eye movements, each antennal movement can be the result of a different mixture of central inputs presynaptic to the antennal motor pool, some of which we have shown to be accessible in the isolated brain preparation.

Comparatively little is known about the significance of antennal movements, by

published accounts there is evidence that the antennae take part in behaviour not only as waterborne vibration-sensitive organs (Taylor, 1975; Tautz, Masters, Aicher & Markl, 1981) but as effectors in defence (Vedel, 1980), and in tactile communication. *Homarus*, for example, has been observed to execute so-called 'antennal whipping' in which they lightly flagellate the chelipeds of other lobsters near them (Solon & Cobb, 1980). Contact by the antennae is detected by the 'whipped' animals mainly through receptors on the chelipeds, and the interaction can lead to patterns of behaviour varying as widely as attack and flight. Antennal movements in such a context are part of neuronal interactions involving large portions of the central nervous system, and the present study represents only the first stage in the process of unravelling the hierarchy of central control.

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