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INTERNEURONES IN CRAB CONNECTIVES (*CARCINUS MAENAS* (L.)): GIANT FIBRES

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SUMMARY

Five interneurones with cell bodies and dendritic trees in the brain have axons 40–60 μm diameter in one oesophageal connective. The fibres are phasic and multimodal, responding to visual and tactile stimuli. They have complex adaptation properties and two are suppressed completely during certain movements of the animal. The role of the fibres in overt behaviour has not been revealed by electrical stimulation or by examination of output in free walking animals. Several smaller interneurones in the connective are briefly described anatomically and physiologically.

INTRODUCTION

There has been little physiological analysis of the central nervous system (C.N.S.) of crabs, although the behavioural and morphological diversity of the species suits them to comparative studies. Behaviour patterns of crabs are well known. Bethe (1897*a*, *b*) described many elements of stereotyped behaviour in *Carcinus*, including eye and antennae withdrawal, optomotor reactions, the rearing reflex (Aufbaumreflex), the tetanus reflex, the egg protecting reflex, the righting reflex, sideways and forward walking, swimming, defence reflexes, cleaning, copulation and feeding. Furthermore he investigated the dependence of these actions on various sense organs or parts of the C.N.S. by removing or inactivating these structures. Many of these activities can take place in the absence of the brain and other ganglia (Schone, 1961; Cohen & Dijkgraaf, 1961; Wiersma, 1961; Lochhead, 1961). Only the eye reflexes (optomotor, compensatory and withdrawal) and locomotion have been studied in detail physiologically. The locomotion studies have not progressed beyond peripheral analysis of input and output in the thorax and the eye-reflex studies still lack knowledge of the interneurones involved (see review by Wiersma & Fiore (1971) on the optomotor reflex, Sandeman & Okajima (1972, 1973*a*, *b*) on compensatory reflexes, Sandeman (1964, 1967, 1969*a–c*, 1971) on eye withdrawal and reviews by Evoy & Cohen (1971) and Barnes (1975) on locomotion).

Interneurones have been extensively studied in the optic tract of *Podophthalmus* and *Carcinus* (See Wiersma, 1970), but there have been few studies of interneurones in the oesophageal connectives of crabs. Bethe (1897*a*, *b*) describes several nerve cells with axons in these connectives, from methylene blue studies. Horch (1971)

describes responses in the connectives of *Ocypode* to airborne sounds and Roye (19[illegible] describes several units in *Callinectes* connectives with phasic statocyst input.

The practicality of studying interneurones in crab connectives has been demonstrated in related decapods. In the crayfish, many large interneurones have been shown to have constant, often relatively simple sensory fields, and constant positions in the connectives. This has allowed detailed mapping in the oesophageal connectives (Wiersma, Ripley & Christensen, 1955; Wiersma, 1958; Wiersma & Mill, 1965), in the connectives between thoracic and abdominal ganglia (Wiersma & Bush, 1963) and between abdominal ganglia (Wiersma & Hughes, 1961). None of the activity of these cells appears as overt behaviour, and stimulation of single interneurones produces no external effect. Their output on other elements 'nearer' the motor side has been observed in certain cases (Kennedy, 1971; Zucker, Kennedy and Selverston, 1971; Zucker, 1972*a–c*). Other cells which evoke complex sequences of muscular contraction and inhibition on stimulation (command fibres) have been demonstrated in the crayfish connective (Atwood and Wiersma, 1967). The crayfish giant fibre system provides one of the best examples of a command fibre system (see Kennedy, 1971; Zucker, 1972*a*, *b*, *c*).

The present study investigates the special features of interneurones in crab connectives which may be relevant to the behaviour patterns mentioned in the first paragraph. This paper is based on a study of a conspicuous group of five large fibres (1–5, Fig. 14, Plate 1) and a sixth large element (6, Fig. 12 and Fig. 14, Plate 1) found in each connective. These were studied because their large size facilitates physiological and anatomical analysis. In the course of the study, several smaller fibres were found and their anatomy and physiology where known is given here. Five interneurones with input from the statocyst, including one of the large ones (5, Fig. 12 and Fig. 14, Plate 1), are described elsewhere (Fraser, 1974).

MATERIALS AND METHODS

Animals

Carcinus maenas (L.) were obtained from the University Marine Biological Station, Millport, Scotland, and kept in circulating aerated sea water. Both male and female crabs of carapace width 6·5–9·0 cm were used throughout.

Electrophysiology

A portion of the dorsal carapace corresponding to the protogastric and mesogastric areas was carefully removed leaving the dorsal endothelium intact. The crab was then clamped in sea water between perspex jaws on either side of the broadest part of the carapace. The dorsal endothelium on one side was then cut away, care being taken to keep the median aorta intact. The stomach contents were sucked out with a syringe and the antero-superior dilator and anterior gastric mill stomach-muscles of the exposed side cut. The whole exposed part of the stomach was hooked back, and cooled aerated *Carcinus* saline (Pantin, 1948) was allowed to drip into the exposed area. The oesophageal connective was then cleared from overlying connective tissue and muscle. It was then either cut, and extracellular records made from the cut end with a suction electrode, or put under tension with a glass hook to allow microelectrode penetration (Fig. 1).

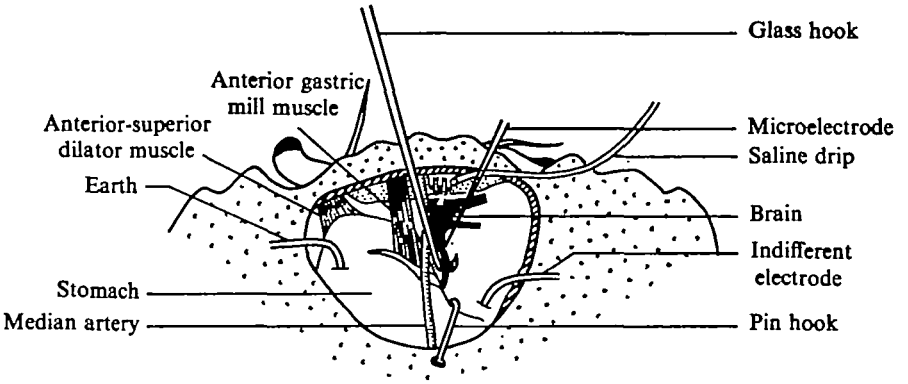


Fig. 1. Recording from the connective. The stomach muscles on the right side have been pulled back with a hook. The connective is steadied by means of a glass hook, and a microelectrode is pushed through the sheath. A saline drip keeps the exposed area moistened and compensates for blood loss.

Dye-filled electrodes containing 4% (w/v) Procion Yellow M-4 or 1 M cobaltous chloride were used throughout. The connective was never desheathed and penetration of the sheath usually broke the electrode tips slightly. After sheath penetration, resistances were typically 15–20 MΩ for Procion and 5–15 MΩ for cobalt. Extracellular records from the fibres in free-walking animals were obtained using implanted pin electrodes. The animal was opened up as before, the electrodes inserted into the connective, and the hole in the carapace sealed with wax anchored with a paper-clip. A detailed procedural account is given elsewhere (Fraser, 1973).

Amplification and display of nerve signals were conventional. Electrical stimulation was carried out using a Grass S8 Stimulator.

Qualitative visual and tactile stimulation was carried out using a spotlight (giving 1250 lux at the animal's eye) with camera shutter, the observers hand, a seeker or a small paintbrush. A photoresistor was used to monitor visual stimuli.

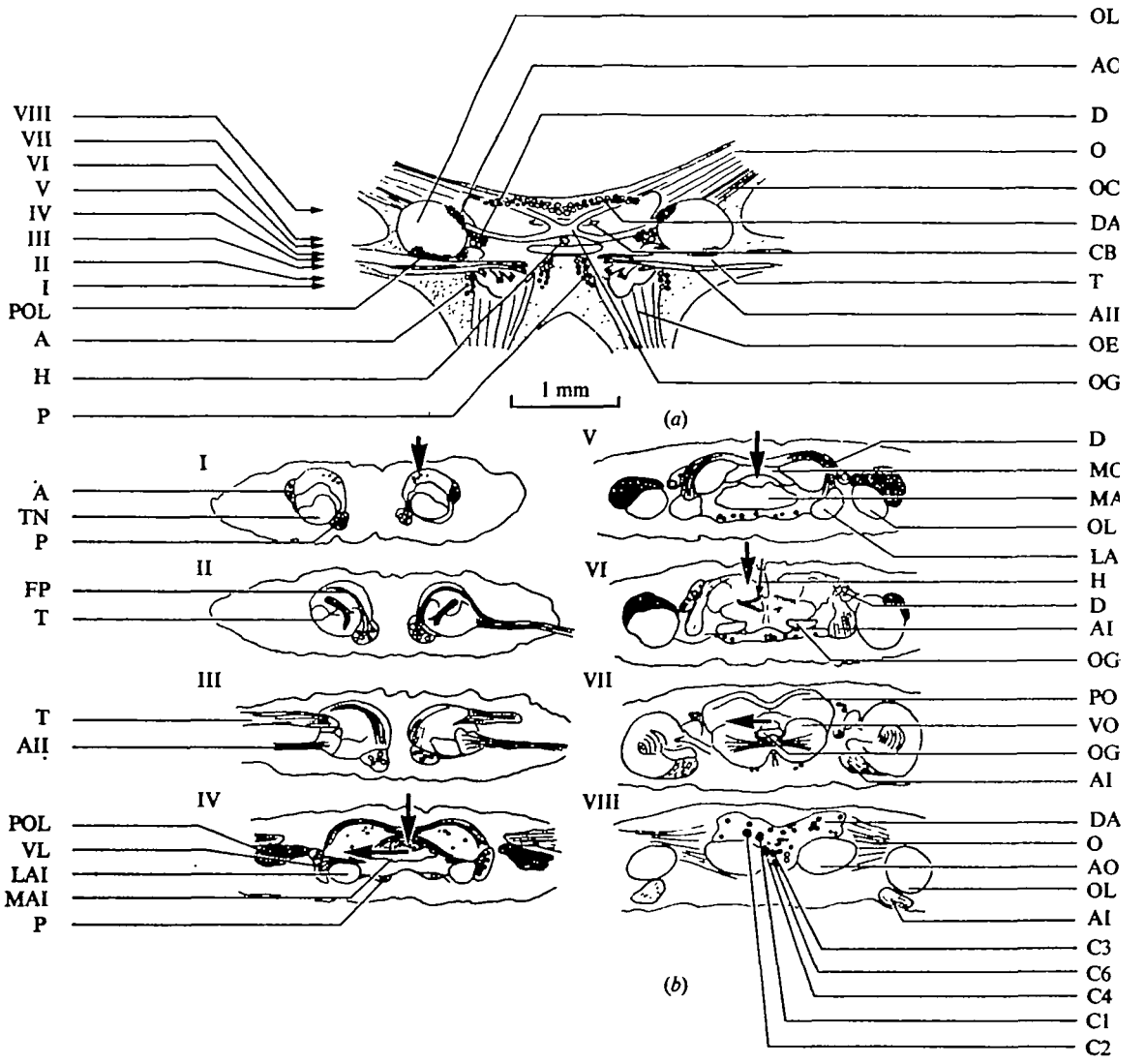
Histology

Brains and connectives were examined in the light microscope after fixation in Duboscq–Brazil and staining with haematoxylin and Light Green. Sections were cut at 24 m. Frozen sections of fresh material were cut at 25 m to check against distortion.

For E.M. examination the connective was fixed *in situ* in 1% OsO_4 in S-collidine buffer at pH 7·4 for 10 min, then cut into pieces 1 mm long and put into fresh fixative for 45 min. These were dehydrated in ethanol and mounted in TAAB embedding resin. Staining with 5% uranyl acetate was carried out at the 90% ethanol stage. Thin sections at 900 A° were examined on Formvar-coated slot grids, enabling the whole cross-section of the connective to be photographed for an axon count.

Dye injection

Procion Yellow was injected electrophoretically using 0·5 sec, 2 μA pulses of current (in a manner similar to that used by Stretton & Kravitz, 1968), into the axons of fibres in the connective for $\frac{1}{2}$ h. It was then left to diffuse for 8–12 h before *in situ*



I–VIII	Levels of sections I–VIII shown in b.
POL	Posterior olfactory cells.
A	Angular cells.
H	Hole for cerebral artery.
P	Posterior cells.
TN	Tegumentary neuropile.
FP	Tract of fibres from posterior cells.
T	Tegumentary nerve.
AII	Antennary nerve.
VL	Ventral lateral cells.
LAI	Lateral antennulary neuropile.
MAI	Median antennulary neuropile.
OL	Olfactory lobe.
AO	Anterior olfactory cells.
D	Dorsolateral cells.
O	Optic tract.
OC	Oculomotor nerve.
DAM	Dorsal anterior medial cells.
CB	Central body.
OE	Oesophageal connective.
OG	Olfactory-gobular tract.
MO	Median oculomotor neuropile.
AI	Antennulary nerve.
PO	Posterior optic neuropile.
VO	Ventral optic neuropile.
AON	Anterior optic neuropile.
C 1–4	Cell bodies 1–4.
C6	Cell body 6.

Fig. 2. for legend see opposite page.

fixation in Duboscq–Brasil. Sections at 25 μm were examined by fluorescence microscopy. 1 M cobaltous chloride was injected for 2 h with a current of 2 μA and developed and fixed as described by Pitman, Tweedle & Cohen (1972).

RESULTS

Brain anatomy

The description of *Carcinus* brain by Bethe (1897*a*, *b*) summarized by Bullock & Horridge (1965) confuses the antennary and tegumentary neuropiles. Furthermore, the accessory lobe reported in Bullock and Horridge to be absent is in fact a small round lobe described by Bethe on the ventral side of the olfactory lobe, just posterior to the olfactory–globular tract. Fig. 2 illustrates some of the major components of the brain, and in particular shows the projections of the tegumentary and antennary nerves. Only these projections are described in detail here. Adequate details of the rest of the brain are given in the references above. Terminology is as recommended in Bullock & Horridge.

Antennary and tegumentary projections

The tegumentary nerve is thicker than the antennary nerve and enters dorsally. A branch containing large axons plunges down and slightly forward towards the ventral neuropiles. Another branch containing large axons goes anteriorly towards and across the ventral midline where it probably joins the oculomotor neuropile. The main portion of the nerve containing fine fibres fans out posteriorly in two main branches to the hindmost neuropiles of the brain which Bethe (1897*a*) described as antennary neuropiles, and referred to here as the tegumentary neuropile.

The antennary nerve enters ventrally into a neuropile at its point of entry on either side. A branch is also sent dorsally over the connective fibres, posterior to the tegumentary nerve and supplies a more medial part of the neuropile.

Fig. 2. (*a*) Diagrammatic horizontal plan of the brain (dorsal view) showing major nerves, cell masses and tracts. The antennulary nerves enter ventrally. The projections of tegumentary and antennary nerves are shown. The arrows 1–8 indicate the levels shown in transverse section in (*b*).

(*b*) Camera lucida drawings of transverse sections at the levels indicated in (*a*).

(I) Tegumentary neuropile, angular cells and posterior cells. The giant fibres 1–5 (arrow) and fibre 6 are still occupying their usual positions in the connective (see Fig. 12. and Fig. 14, Plate 1).

(II) The tegumentary nerve entering the tegumentary neuropile. Fibres 1–3 pass medially to the tract of fibres from the posterior cells.

(III) The antennary nerve entering and fanning into the antennary neuropile. The first lateral branches of fibres 1–4 have been given off and run dorsally over the exiting fibres of the tegumentary nerve.

(IV, V) The giant fibres 1–4 from both sides come together into a median tract (vertical arrow). Crossing over to the other side of the brain occurs in this tract and branches are sent into the median oculomotor and median antennulary neuropile. The horizontal arrow in Fig. 3*b* and 4 shows the tract occupied by branches of fibres 2, 3 and 4 crossing to the contralateral antennal and tegumentary neuropiles.

(VI) Fibres 1–4 have now crossed over the midline and run anteriorly in the tract indicated by the large arrow. Fibre 6 crosses over in the tract of fibres indicated by the small arrow.

(VIII) Posterior protocerebrum, the horizontal arrow indicates the level of the contralateral branches of fibres 1, 2 and 4. Ipsilateral branches into the optic neuropiles run just above the ventral optic neuropile.

(VIII) The approximate levels of the cell bodies of fibres 1–4 and 6 are indicated.

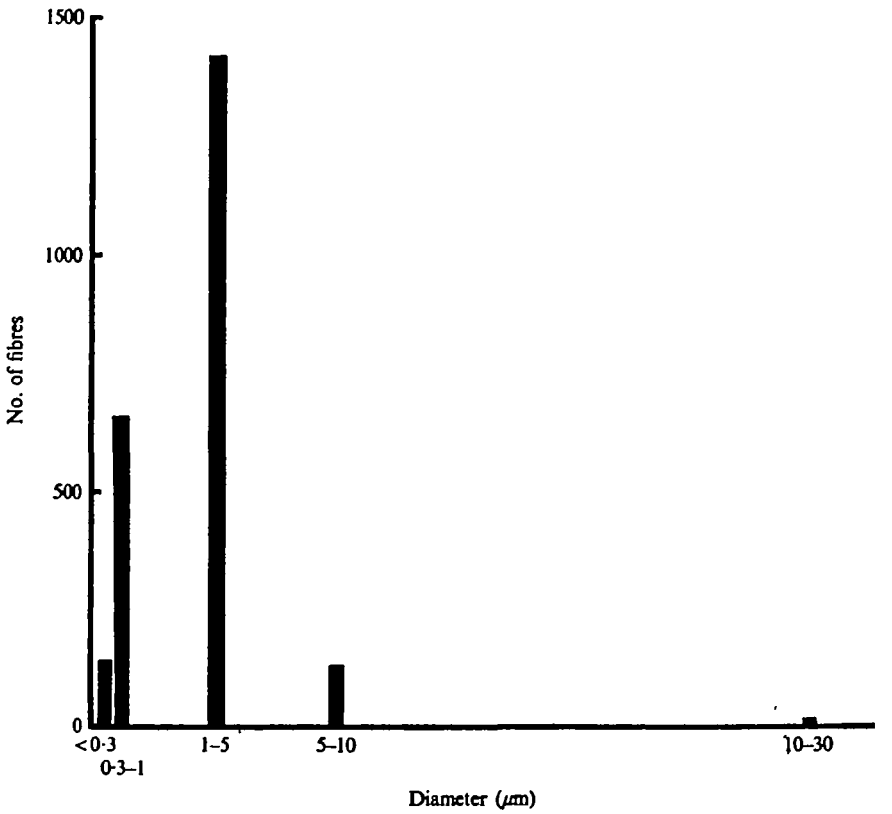


Fig. 3. Size distribution of fibres in *Carcinus* connective from E.M. count.

Diameter (mm) ...	<0·3	0·3–1·0	1·0–5·0	5·0–30·0	10·0–30·0	Total
No. of fibres ...	145	654	1417	128	15	2359

Connective anatomy

The general form of the connective can be seen from Fig. 12 and Fig. 14, Plate 1. A montage of E.M. photographs allowed counts of all the fibres in the connectives. There are about 2300 fibres. Histograms of axon diameters were obtained from one of three counts (Fig. 3). Considerable shrinkage occurred during E.M. processing, so the size distribution figures are displaced to the left. A more accurate picture of the large fibres is obtained from material fixed for light microscopy and from fresh tissue cut in a cryostat (Fig. 4). There are typically 40–50 fibres over 10μm diameter in each connective.

The largest fibres (1–5, Fig. 12 and Fig. 14, Plate 1) always occur in a characteristic group. Their relative positions in the group vary from animal to animal, but are constant in any one animal down the entire length of the connective, and into the thoracic ganglia. A sixth fibre (6, Fig. 12 and Fig. 14, Plate 1), on the outside of the connective, is similary variable in its relationship to neighbouring small fibres. Fibres in the left connective are roughly mirror images of those in the right connective. These six fibres on each side are called giant fibres by analogy with other large interneurone systems

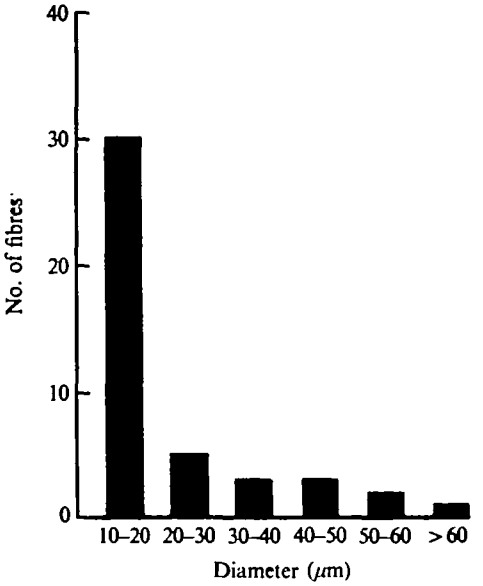


Fig. 4. Size distribution of larger fibres from Duboscq-Brasil fixed material. Average of six crabs.

Diameter (μm)	10–20	20–30	30–40	40–50	50–60	> 60	Total
No. of fibres	30	5	3	3	2	1	44

Anatomy of giant fibres

All giant axons (1–6, Fig. 12 and Fig. 14, Plate 1) were penetrated intracellularly and filled with Procion Yellow and cobaltous chloride. The plans given here are camera lucida drawings of the best cobalt injections of each in whole mounts. Fig. 5 shows cells 1–4 and 6. Cell 5 is the subject of a separate investigation (Fraser, 1974), together with other interneurones which have input from the statocyst.

All the cell bodies are large (40–60 μm) and lie contralaterally in the dorsal anterior medial cells. The major processes lie together and run in definite tracts between neuropile masses.

Cells 1–3

Cells 1–3 run close together for most of their length. They each give off an ipsilateral branch before the main axon narrows in the brain. These branches run dorsally over the connective fibres and then just above the tegumentary nerve where it enters the neuropile. Fine processes from these branches lie in the tegumentary and antennary neuropiles but most of the larger processes of these branches lie above the fine-fibred neuropile. The main parts of the three cells pass medially to a tract of fibres from the posterior cells and then run in the tract shown in Fig. 2*b*, IV and V, cell 3 lying ventral in the tract, cells 1 and 2 lying more dorsal. The cells continue in this tract, crossing over the midline and sending branches into the median oculomotor neuropile. All these fibres send a branch into the ipsilateral ventral and median optic neuropiles. Cells 2 and 3 send a branch in the tract shown in Fig. 2*b*, IV, between the oculomotor and antennulary neuropiles. Finer ramifications of these branches run in the antennary and tegumentary neuropiles. Cell 2 also sends branches into the median antennulary

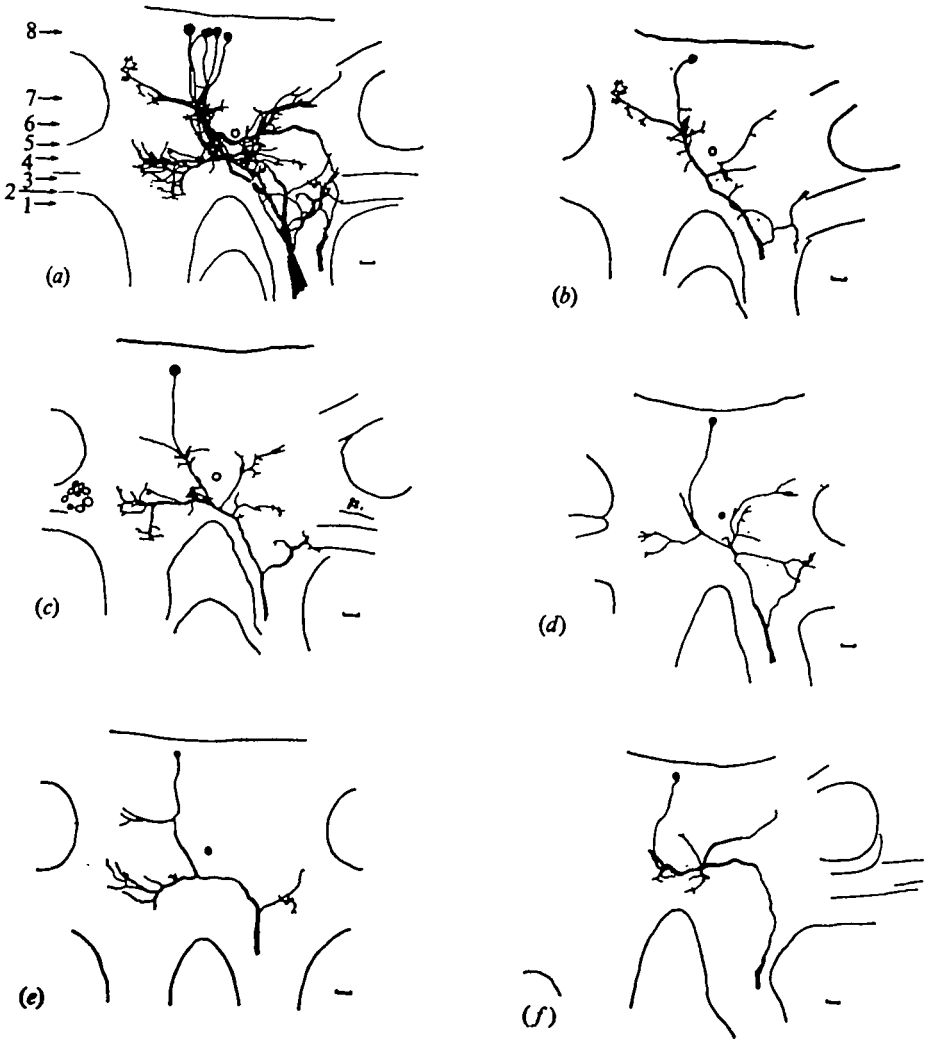


Fig. 5. Camera lucida diagram of cobalt injected fibres 1–4 and 6 in the brain. Features of the brain which were visible are included. These should be compared with Fig. 3. The approximate levels of the sections shown in Fig. 3*b* are indicated in (*a*). (*a*) All five fibres, 1–4 and 6; (*b*) fibre 1; (*c*) fibre 2; (*d*) fibre 3; (*e*) fibre 4; (*f*) fibre 6. Scale 100 μm.

neuropile and the lateral oculomotor neuropile on both sides. Cell 1 has no branches in the contralateral deuterocerebrum but has several processes in the contralateral posterior and median optic neuropiles, and a main branch which runs between the posterior and median optic neuropiles (Fig. 2*b*, VII). Cell 2 has a few fine processes running with those of cell 1 in the contralateral optic neuropile. Cell 3 has a fine branch in the contralateral ventral optic neuropile. The neurites to the cell bodies of cells 1 and 2 run very close together to the dorsal anterior medial cells and then separate. The neurite of cell 3 runs nearer the ventral side and the cell body lies below that of fibre 1, nearer the midline. The approximate positions of all three cell bodies are shown in Fig. 2*b*, VIII.

Cell 4

Cell 4 has its first ipsilateral branch farther forward than fibres 1 and 3 and initially the main fibre runs laterally to the tract of fibres from the posterior cells (Fig. 2*b*, II and III). This branch runs dorsally over the connective axons and tegumentary nerve, and part of it stays amongst fairly thick dorsal fibres in this part of the brain; a second process goes ventrally into the tegumentary and oculomotor neuropiles. The main part of the cell joins the tract of fibres containing cells 1–3. The cell has very few processes in the median oculomotor neuropile, but sends a branch beside fibre 2 with processes in the contralateral tegumentary, antennary and lateral oculomotor neuropiles. There is also a more anterior branch between posterior and median optic neuropiles with processes in the median optic neuropile. The cell body position in the dorsal anterior medial cells is shown in Fig. 2*b*, VIII.

Cell 6

Cell 6 remains lateral to the tract from the posterior cells, keeping to the other side of the connective, until it turns at right angles to run to the other side of the brain in the tract of fibres shown in Fig. 2*b*, VII. Processes are given off into the median oculomotor, and median and posterior optic neuropiles. The neurite to the cell body runs dorsally and then plunges ventrally to the cell body (Fig. 2*b*, VIII).

Electrical activity

Extracellular records with suction electrodes on the brain side of the cut connective showed large spikes in response to visual and tactile stimuli. No similar large spikes were ever evoked from the thoracic side of the connective. Records from two points of an intact connective confirmed that the large fibres were descending fibres. Latencies at 23 °C were light off, 110 msec; light on, 91 msec; touch on the anterior carapace 8 msec.

Records with intracellular microelectrodes filled with dye allowed identification of the separate fibres. Simultaneous suction electrode records and microelectrode records also confirmed that the large spikes recorded extracellularly came from the giant fibres. All the fibres were multimodal.

Cell 1

Cell 1 reacts phasically to movement in the visual field, light off, light on, touch over the whole carapace and on the bases of the head appendages and walking legs. Between stimuli the unit is silent except in very excited animals when an irregular spontaneous discharge is maintained. The response to a sharply defined stimulus, such as light off, typically has spikes grouped into an early burst of one to three spikes and a late burst of one to several spikes (Fig. 6). Exact sensory fields and thresholds were difficult to establish because of variable adaptation. Visual input to either eye gives a response. The response to all these inputs adapts rapidly, but at different rates. Responses to light on and touch on the dorsal carapace habituate easily and are seen only in well-rested animals. Movement in the visual field gives the most persistent response. The output of the fibre is usually suppressed during 'spontaneous' movements of the animals' legs. Suppression of the response to light off is shown in Fig. 7. The fibre is not suppressed during all leg movements. A response is evoked when the

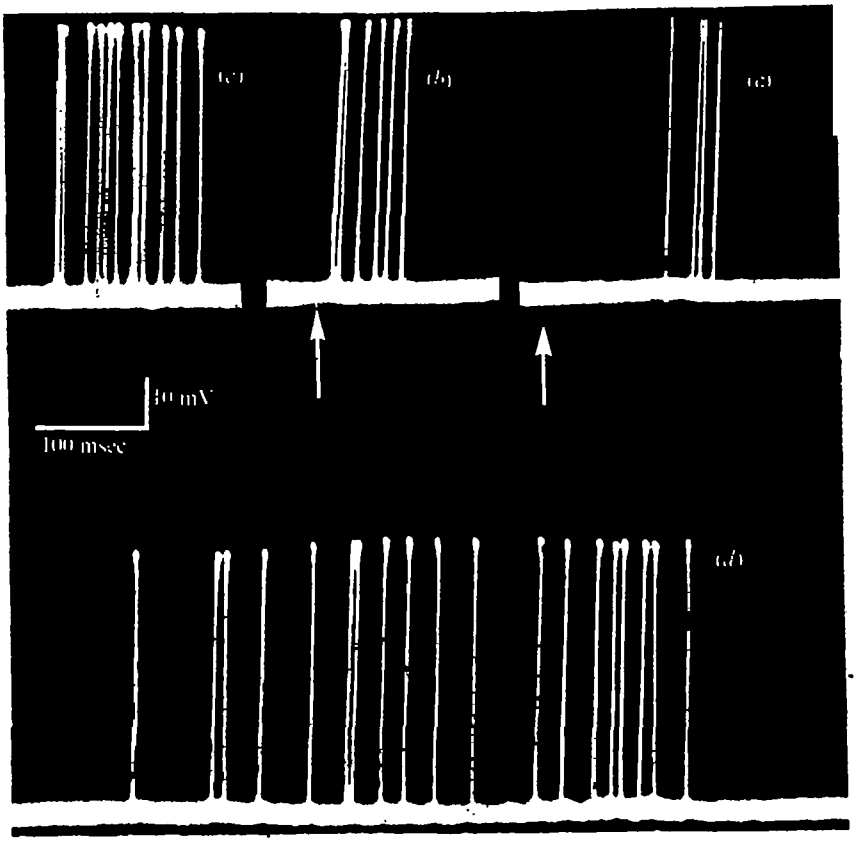


Fig. 6. Intracellular record from Fibre 1. Records go from left to right. (*a*) Light off (arrow); note the single early spike and later burst. (*b*) Touch round the anterior carapace (monitored approximately by the arrow). (*c*) A hand moved over the light source giving an off and a movement component. (*d*) Movement of a hand above the animal.

animal cleans its own eye-socket with its ipsilateral second walking leg. A novel stimulus will often elicit a response from an adapted cell. In one preparation, for example, usually effective tactile and visual stimuli gave at most one to three spikes. However, when the observer raised his head from the dissection microscope there was a single vigorous burst. Repetition of this movement produced no further response.

Cell 2

Cell 2 is the largest fibre in the connective and its activity can be seen in extracellular records as a very large spike during tactile or visual stimulation (Fig. 8). Only one or at most two spikes follow any stimulus, and spikes hardly ever occur to consecutive stimuli even with many minutes between stimuli. The sparse nature of the discharge makes analysis of the sensory fields difficult. It seems to respond to the same modalities as fibre 1, over the same fields, but with a very much reduced discharge rate.

Cell 3

Cell 3 is similar to cell 1. It responds to touch all over (especially the anterior carapace, bases of head appendages and bases of walking legs) and to visual input

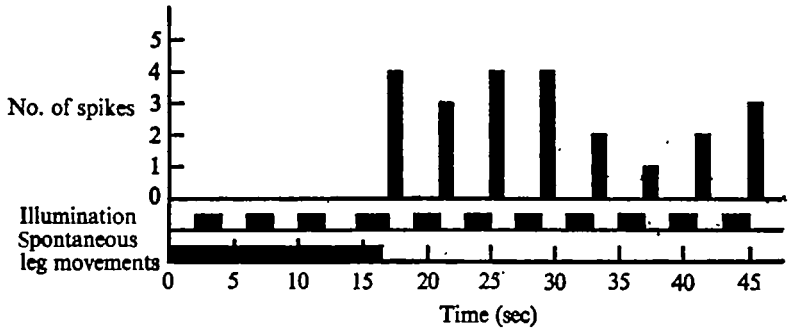


Fig. 7. Inhibition of fibre 1. A light is flashed on and off with a period of about 2 sec. Under these conditions a burst of 3–4 spikes is normally evoked from fibre 1 on light off. During spontaneous leg movements (lower black bar) no response is evoked. Light off is monitored by a black bar.

Fig. 8. Suction electrode record from the whole right connective, showing the response to a tap on the anterior carapace (large arrow). A single large spike from fibre 2 is indicated by the small arrow. Trace goes from left to right.

(movement in both visual fields, light off and on). The most conspicuous input and the slowest one to adapt is touch on the contralateral antenna base. All other inputs adapt very quickly. As with cell 1, output is suppressed during 'spontaneous' movements of the legs. Response levels vary in the same way as described for cell 1.

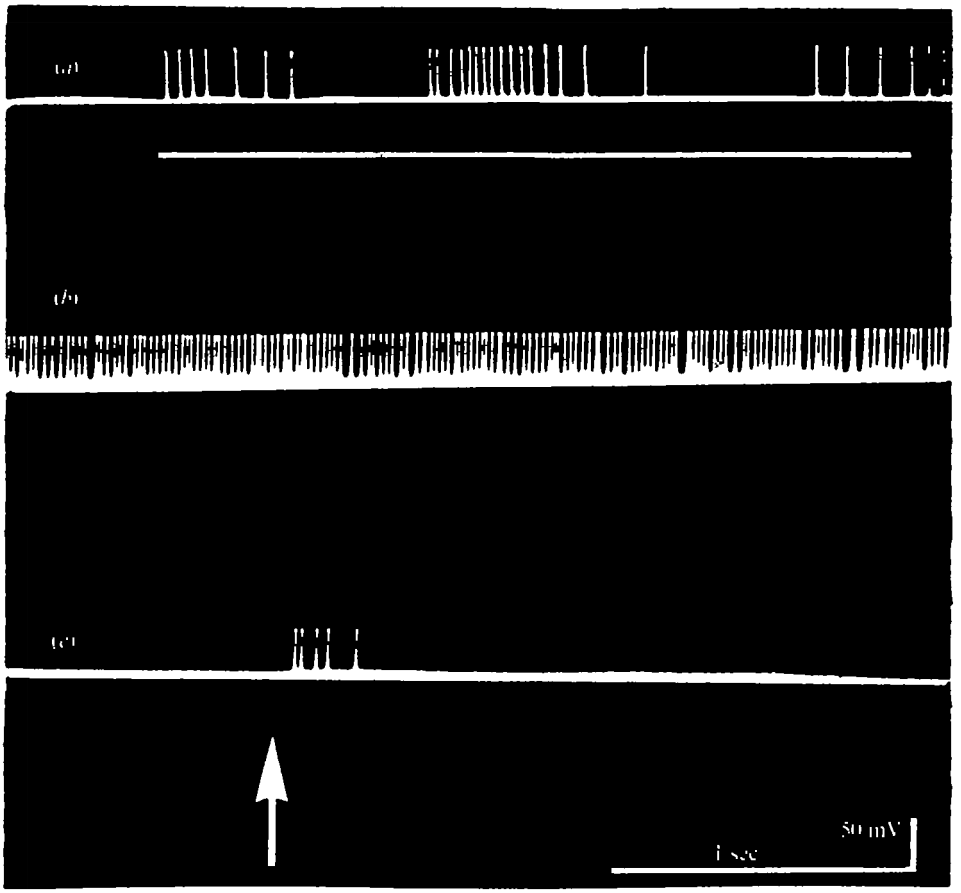


Fig. 9. Intracellular record from fibre 4. (*a*, *b*) Continuous record showing response to tactile stimuli round the head appendages (white bar). Tonic activity persists after the stimulus and carries on for about 20 sec. (*c*) Response when not excited is phasic. Light off (arrow) evokes a short burst of spikes. Traces go from left to right.

Cell 4

Cell 4 reacts to light and touch with sensory fields similar to cells 1 and 3. It differs from these cells by having a sustained tonic discharge on prolonged mechanical stimulation which greatly outlasts the duration of the stimulus (Fig. 9). This discharge is often correlated with 'spontaneous' appendage and leg movements. The fibre usually fires before the overt movements start, and goes on firing after they cease.

Cell 6

Cell 6 characteristically gives a long erratic discharge to; light off, light on, movement in the visual field, and forced movements of the legs upwards round the basal joints (Fig. 10). The cell differs from cells 1–4 by not responding to tactile stimulation of the anterior carapace. It responds to touch only on the legs. Movement in either visual field is the best adequate stimulus. Adaptation of the unit is selective and

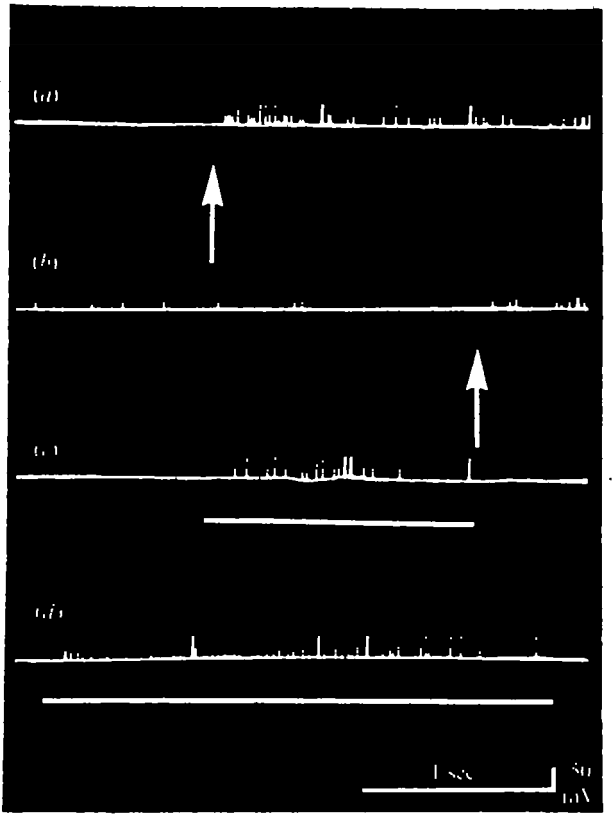


Fig. 10. Intracellular record from fibre 6. (*a*) Response to light off (arrow). (*b*) The end of the off response and the response to light on (arrow). This trace starts 8·5 sec after the end of trace (*a*). The on response continues for another second after the end of this trace. (*c*) Response to movement of a hand in the visual field (roughly monitored by the white bar). (*d*) Response to raising the legs on one side about the basal joints (roughly monitored by white bar). Traces go from left to right.

variable in a manner similar to cell 1. The responses to forced leg movements are least reliably evoked. No inhibition of response during 'spontaneous' leg movements was noted.

Summary of cells 1–4 *and* 6

Although all these cells are multimodal, they are distinguishable physiologically by either their firing pattern or their preferred input. These characteristics are shown in Table 1.

Conduction velocity

Giant axons had conduction velocities of between 5·0 and 7·3 m/sec, and medium-sized ones about 4·0 m/sec, as recorded from two points a measured distance apart on the connective.

Electrical stimulation

Stimulating the giant axons individually up to 200 Hz through the microelectrode in otherwise intact animals, produced no overt behaviour. Simultaneous extracellular

Table 1

Cell no.	Most adequate stimulus	Nature of discharge
1	Movement in either visual field	Phasic
2	Touch on anterior carapace and bases of head appendages. Bilateral	Sparse, 1–2 spikes, difficult to excite
3	Touch on contralateral antenna base	Phasic
4	Touch on anterior carapace and bases of head appendages. Bilateral	Sustained over-shooting regular discharge on vigorous stimulation
6	Movement in either visual field (no tactile input from head regions)	Prolonged erratic discharge to light 'off' or movement.

records confirmed that the axons responded. Stimulation with large currents elicited a variety of complicated but repeatable appendage movements. In all cases current must have spread to more than one axon, and the command fibres concerned are almost certainly smaller than the giant fibres. The sequences obtained on stimulation of particular locations in the connective are described elsewhere (Fraser, 1973). The complete swimming reflex as described by Bethe (1897*a*, *b*) and Hartnoll (1971) could be obtained through localized stimulation in either connective.

Electrical stimulation of sensory axons in the various brain nerves could not be done adequately as dissection near the brain tended to disrupt the blood supply essential for the normal functioning of the giant cells. Such stimulation is not very meaningful anyway, both for sensory nerves and central tracts like the optic tract, as it does not preserve the normal temporal relationships between afferent elements and interneurones which excite the fibres studied, and it must excite many inappropriate elements. Electrical stimulation of sensory nerves was never carried out when recording from single giant cells. It was possible, however, to record from the group of giant cells in the whole connective while stimulating particular nerves. In this way it was shown that all the brain nerves provide input to the giant cells as a group, but they do not all necessarily provide input to each giant cell.

Simultaneous recording from a whole connective and each of the brain nerves in turn showed no correlations between action potentials in giant cells and those of large units in these brain nerves.

Other interneurones

Apart from the giant fibres and the statocyst fibres described elsewhere (Fraser, 1974) various other fibres were recorded and injected with dye. Dye injection into a single axon for periods long enough to fill the cell in the brain often caused filling of several cells due to leakage. Two or more cells often took up the dye, although the microelectrode seemed by physiological criteria to be within a single cell. In the case of the giant cells, sufficient numbers of good penetrations and single injections were made to allow unambiguous correlation of anatomy and physiology. The smaller elements, however, were not penetrated often enough for this. The distribution in the brain of five smaller elements is given in Fig. 11. Four of the fibres have cell bodies ventrally in the posterior cells, and the fifth fibre disappears into the ipsilateral optic nerve.

Dye injection for short periods into axons revealed their location in the connective unambiguously. The fibres detailed below (Table 2) have fairly simple properties

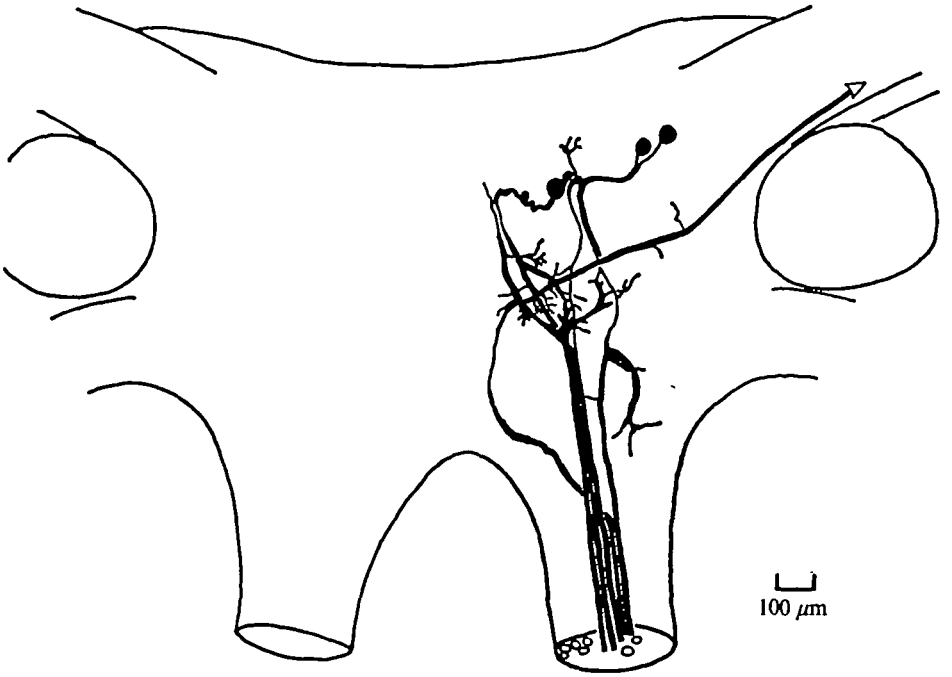


Fig. 11. Camera lucida drawing of five medium sized cells in the brain. The cell bodies shown are all ventral in the posterior cells.

Table 2

Cell no.	Discharge pattern	Unit characteristics
I	Rhythmic	Bursts of 8–20 spikes, ½–1½ sec apart. No input found
II	Rhythmic	Bursts of 6–20 spikes, 2–3 seconds apart. The number of spikes per burst was reduced in the light or by tactile stimulation about the anterior carapace
III	Rhythmic	Bursts of 2–4 spikes, 1–2 sec apart. No input was found
IV	Tonic	Frequency increased on light off and decreased slightly on light on. Inhibited altogether during movements of a large object in the visual field. Tactile stimulation of the anterior carapace increased frequency (Fig. 13)
V	Tonic	Frequency increased on light off and by touch all over the carapace.
VI	Tonic	Sustained discharge correlated with movements of the legs. Frequency increased on touching legs or claws.
VII	Irregular tonic	Apparently inhibited by light. No adequate stimulus could be found
VIII	Rhythmic	Bursts of 8–10 spikes every 1½ sec. Rhythmic discharge was inhibited by repeated light on light off, then the fibre reacted phasically to movement in the visual field, light off and touch over the anterior of the animal.
IX	Tonic	Responded with overshooting response to movement in the visual field and touch round the anterior carapace.

and their location in the connective is accurately known (Fig. 12). In general the complex inputs to the fibres and differences in responsiveness to various stimuli which occurred naturally and on deterioration of preparations made them difficult to categorize.

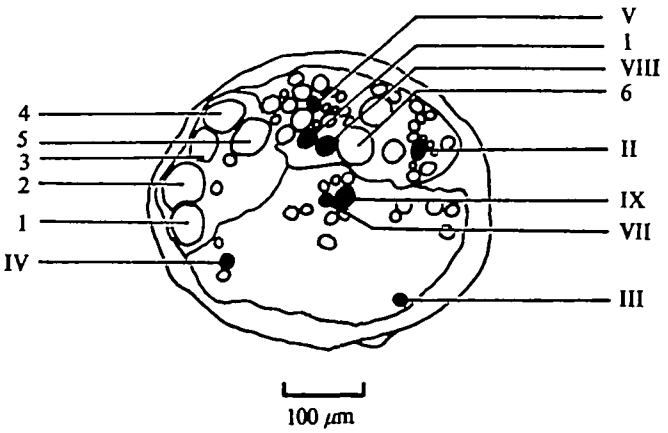


Fig. 12. Location of fibres I–IX in a camera lucida diagram of a connective. Fibres 1–6 are shown for reference.

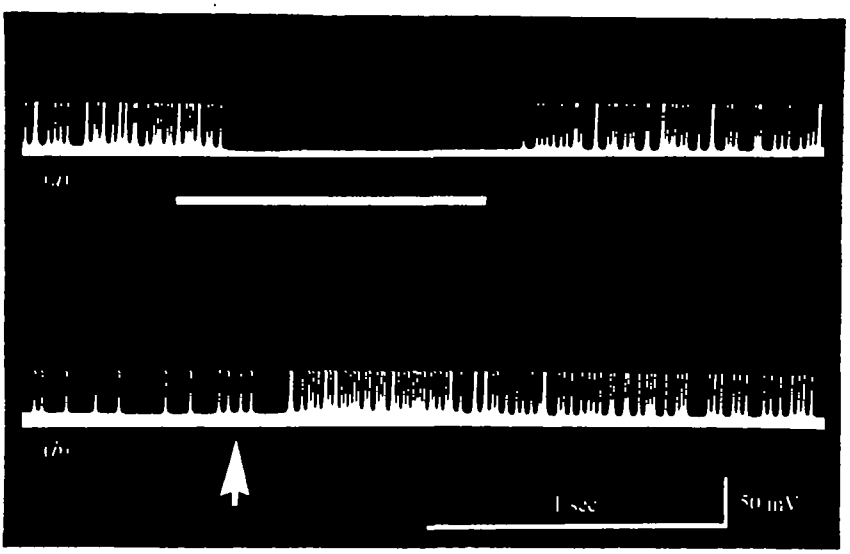


Fig. 13. Intracellular record from fibre IV showing (*a*) inhibition of the unit during movement of a hand in the visual field (monitored roughly by the white bar), (*b*) increase in frequency to light off (arrow) – traces go from left to right.

Free-walking preparation

Seven successful free-walking preparations were made, allowing extracellular records of the connective fibres to be made with the crab behaving quasi-normally. It was difficult to distinguish all giant spikes unambiguously, but the separate large units could be recognized by their characteristic discharges.

In general, in the free animal all responses were more readily evoked than in the restrained crab. Visual responses were more sustained, adapting far less rapidly. Activity of the contralateral antenna fibre (fibre 3) was very obvious, and there seemed to be more large spikes from axon 2 in response to tactile or visual stimulation. During states of high excitement there was activity from at least two large fibres.

No obvious correlation of any of the units with behaviour was observed. The full rearing reflex could be evoked without conspicuous activity in giant fibres. However, when a large object approaches the animal, it rears up, visually tracking the object to keep its claws in the optimum position. During this part of the reflex there was giant fibre activity seemingly correlated with the tracking movements. The large fibres certainly do not command the basic reflex (which is a tonic reflex). There is nearly always a burst of giant fibre activity on eye withdrawal, but no activity during cleaning of the eye with the ipsilateral third maxilliped. There was no correlation observed between giant fibre activity and withdrawal of any of the mouthparts, although this was looked for many times because mouth-part withdrawal is the most stereotyped behaviour caused by stimuli which fire the giant interneurones. Any apparent correlation was never rigid and could be explained by parallel input. Rhythmic bursts of giant fibre activity occurred during sideways walking.

In general the units all responded to the stimuli that were adequate in restrained crabs. Differences in excitability fell well within the expected range found in the restrained animals. No special inputs were revealed by increasing the animals' freedom.

DISCUSSION

The results presented here cannot easily be fitted into a behavioural framework. On the input side of these interneurones, broad sensory fields have been established but the receptors or interneurones responsible remain unidentified (see Kennedy, 1971). On the output side, nothing has been established. The interneurones spread widely over neuropiles in protocerebrum, deutocerebrum and tritocerebrum, being absent only from the optic lobes in the eyecup, olfactory and accessory lobes, central body and protocerebral bridge, which according to Maynard (1965) are the likely sites of complex and long term behaviour. Comparison of elements in the crab with those of other arthropods may help in our understanding of arthropod behaviour at the cellular level but true homologies are difficult to establish.

Giant interneurones in the Brachyura

This is the first time that giant interneurones have been described physiologically in the Brachyura. Their occurrence in *Carcinus* and also as identical groups in *Macropipus*, *Cancer* and *Scylla* (Fraser, 1973 and unpublished observations) suggests they are important throughout the Brachyura. Giant fibres have been noted anatomically by Skobe & Nunnemacher (1970) in Brachyura. Their measurements of *Carcinus* elements do not allow for shrinkage during fixation. The difference in the number of fibres in *Carcinus* connective found in their study (2767) and in this study (2300) may reflect individual variation or degeneration of certain fibres.

Output of giant fibres

No overt behavioural output for the crab giant fibres is known. In this respect they resemble large interneurones in the cockroach (reviewed by Parnas & Dagan, 1971), locust (descending movement detector – DMD – neurones reviewed by Rowell, 1971; O'Shea & Rowell, 1974) and in fact most interneurones, but differ from crayfish medial and lateral giant fibres which evoke tail flips (Wine & Krasne, 1972).

An output for locust DMD fibres has been found (Burrows & Rowell, 1973). Excitatory post-synaptic potentials (EPSP's) directly correlated with spike activity in these DMD units have been shown in thoracic motoneurones involved in the back-leg kick. These EPSP's were never sufficient to cause spike activity in the motoneurones and therefore are only visible as overt behaviour in conjunction with other nerves or in unrestrained insects. It may be that crab giant interneurones have a similar subthreshold output and are only part of a functional group of elements necessary to initiate and maintain a behaviour pattern. Lack of correlation of single interneurone activity with behaviour in the free walking animal is not surprising. Interneurones may only participate during one part of a behaviour pattern and several driving systems for a given behaviour pattern are normal. Thus escape swimming in the crayfish is driven by the giant fibres for the first tail flip and then by non-giant interneurones. Not all swimming involves giant-fibre activity. Only giant fibres in one connective of the crab were monitored in this study, but homologous elements in either connective of arthropods usually participate identically in the same behaviour (e.g. Burrows & Rowell, 1973; Kennedy, 1971), activity of both homologues must be considered in the correlation of the elements with behaviour.

Input to giant fibres

The optimum stimulus for the crab giant interneurones is likely to be some subtle combination of visual and tactile input which has almost certainly not been hit upon by the experimenter. An understanding of this optimum stimulus is unlikely to be reached without knowledge of the output. Short latencies for tactile stimuli suggest a fairly simple input cluster involving no more than first- or second-order interneurones, whereas long latencies for visual stimuli suggest the involvement of high order fibres. The response to a single stimulus (e.g. light off, Fig. 6) consists of two bursts of spikes, and on many occasions several bursts were noted. This is a common phenomenon in interneurones and has been shown by Zucker (1972*a*) in the crayfish lateral giant fibre to be due to an input arrangement whereby afferents excite the high order interneurone directly and also by slower, less direct, pathways. Similar input chains are likely to exist for the crab fibres. In the case of repetitive bursts caused by visual stimuli, no multiple projections of afferents (which end in the lamina) are possible, and input chains must involve interneurones only.

Anatomy of giant fibres

Knowledge of the anatomy of the giant fibres is useful in terms of their identification, but as yet yields no insight into input or integrative mechanisms. Anatomical descriptions of arthropod brains partition them into discrete neuropiles named after the sensory nerves which end in each, structured neuropiles, definite tracts and separate groups of cell bodies. This crude description is useful in providing landmarks but the described neuropiles do not even form a complete plan of sensory destinations. Thus sensory fibres from tegumentary, antennary and oculomotor nerves project to the median oculomotor neuropile, while sensory and low-order interneurones from the thoracic centres have no named projection in the brain, although it is known that they reach the optic lobes in the eye-cup (Bush, Wiersma & Waterman, 1964). Anatomical localization of the activity within a ganglion is a reality

only at the single cell level because of the extensive branching of cells. Regional localization of function in an anatomically named region is not demonstrated, although still possible where interneurones of one class are grouped. Maynard (1965) was aware of this problem and suggested a functional division of the lobster brain into three regions. The distribution of the giant interneurone branches supports this division because they are excluded from Maynard's postulated site of complex and long term behaviour (see above). The cell bodies of the giant cells are all contralateral in the dorsal anterior medial group of cells. This group is heterogeneous, containing many elements supplying the optic lobes in the eye-cup as well as the thoracic centres. No correlation of cell body location with axon location is thus possible.

None of the neurones described here anatomically closely resemble the ones described by Bethe (1897*a*) from methylene blue studies, although some of his cells are large. This is not surprising as dye injection is strongly biased towards large cells whereas methylene blue is not, and only small numbers of cells were sampled in both studies.

Comparison of giant fibres in crabs and other arthropods

Points of similarity such as large size, high conduction velocity, phasic nature and response to stimuli threatening to the animal, link cockroach giant fibres, locust DMD fibres and crab giant fibres, but these may only point to those features of interneurones which facilitate study rather than common features of homologues. Cockroach giant fibres (not yet individually identified) are thought to be second-order fibres, near the sensory side, whereas locust DMD fibres excite motoneurones and are hence near the motor side. Each neurone is adapted over a long period to work with its fellow neurones in its own animal, so that comparisons at this level are less likely to be fruitful than comparisons of homologous interneurones in crabs exhibiting diverse behaviour patterns and life-styles.

Other interneurones

In this study five giant fibres are studied as identified fibres. There are, however, about 40 other large elements in each connective, of which 5 are described here anatomically, and 9 are briefly described physiologically. In the following paper (Fraser, 1974) five of these fibres are described physiologically, two of them anatomically. The overall structure of the connective seems to be similar to that of the crayfish (Wiersma & Mill, 1965). Few generalizations are possible regarding these other large elements except that they are mainly descending with cell bodies in the brain, usually multimodal with complex sensory fields, and often exhibit spontaneous rhythmic activity.

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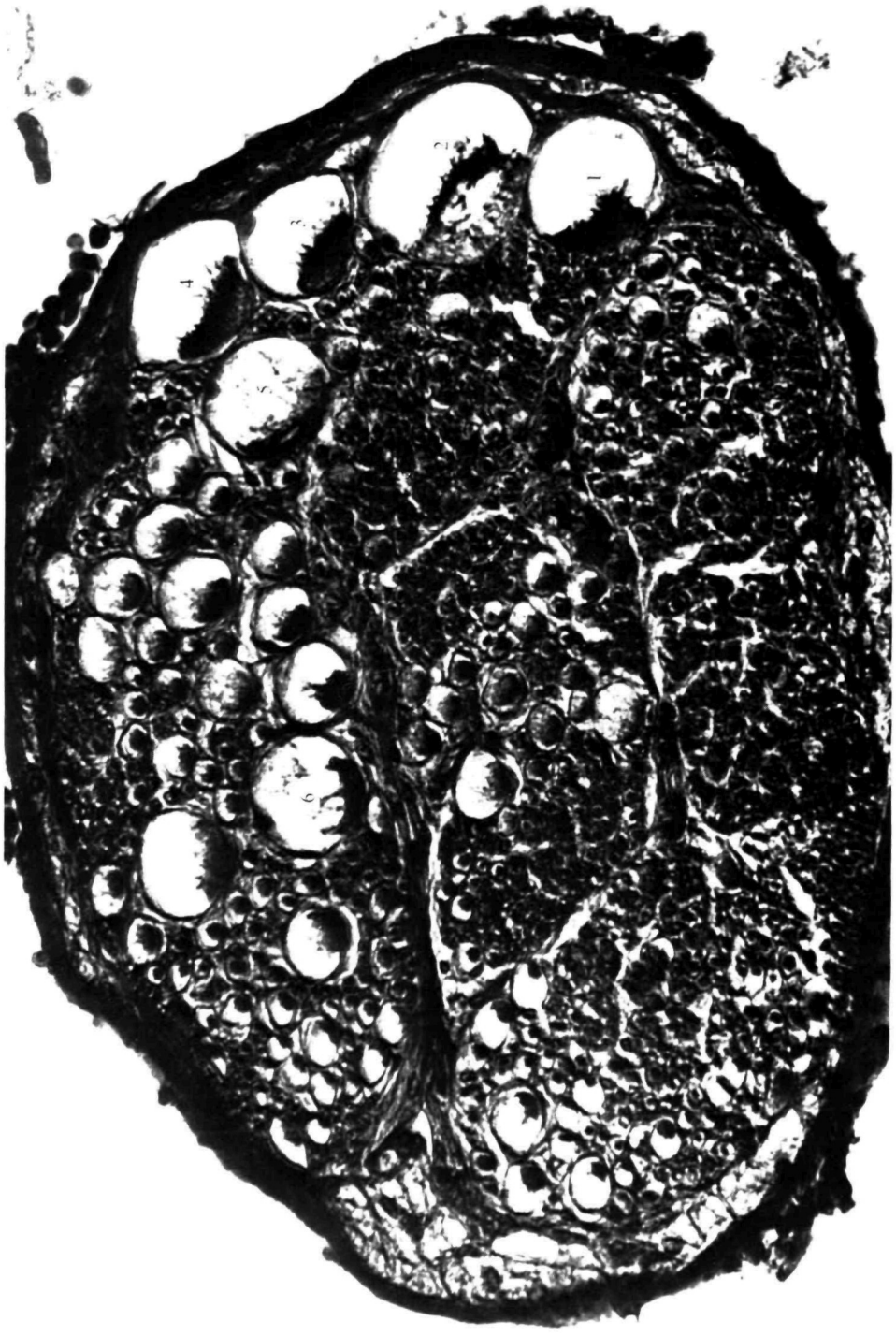
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EXPLANATION OF PLATE

Fig. 14. Transverse section through a left oesophageal connective of *Carcinus* showing the giant fibres.