J. Exp. Biol. (1971), **54**, 391–402
With 2 plates and 5 text-figures
Printed in Great Britain

THE DIFFERENT CONNECTIONS AND MOTOR OUTPUTS OF LATERAL AND MEDIAL GIANT FIBRES IN THE CRAYFISH

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(*Received* 8 *September* 1970)

INTRODUCTION

The giant fibres of crayfish comprise a pre-motor system that secures rapid contraction of phasic flexor muscles in the abdomen. The result of their activation is a sudden flexion of all abdominal segments that propels the animal backward in an 'escape' response; repeated sequences of similar tail-flips, with intervening fast extensions, result in sustained swimming.

The adequacy of single medial or lateral giant fibres for producing such tail-flips was demonstrated by Wiersma (1938) who isolated axons in the circumoesophageal connectives and stimulated them. He also (1947) showed that either kind of central giant produced short-latency discharge of flexor motor neurones in the abdominal third roots. It was not clear from Wiersma's results, however, whether the actions of the two types of giant fibres were really equivalent. It is known that medial and lateral giants differ both in their morphology and in the location of presynaptic input. Medial giant fibres (MG) are single cells whose somata are located in the brain; branches and a cross-connexion also occur there (Horiuchi, Hayashi & Takahashi, 1966), but the axons run caudally without branches or septa to the last abdominal ganglion. Lateral giants (LG), on the other hand, consist of segmental units linked end-to-end in each ganglion by septa across which electrical propagation occurs (Kao & Grundfest, 1956; Watanabe & Grundfest, 1961). A contralateral soma and dendritic branches were shown to occur in the third abdominal ganglion (Remler, Selverston & Kennedy, 1968). MG are said to be excited by stimuli applied to the head region (Wiersma, 1961), whereas the LG are discharged by afferents entering the abdominal ganglia via the second roots (Krasne, 1969).

The experiments we shall describe show that the two types of giant fibres differ distinctively in their motor output, as well as in their input. MG produce a full, symmetrical flexion of the abdomen without flaring of the uropods, whereas LG evoke a flexion in which intermediate abdominal segments are relatively uninvolved and the uropods are strongly promoted. These differences, which are readily seen in the behavioural response to giant-fibre stimulation in the intact animal, are accounted for by specific connexions made by the giant fibres with motor neurones innervating the different muscles involved.

METHODS

Crayfish (*Procambarus clarkii*) were prepared in a variety of ways to gain access to the giant fibres for different purposes. In behavioural experiments the objective was to stimulate medial and lateral giants selectively with minimal interference to the abdomen. They were therefore isolated in the circumoesophageal connectives, which were exposed by removing the rostral-dorsal portion of the carapace and the underlying hepatopancreas and stomach. During this dissection and subsequent recording sessions the animal was immersed in cold van Harreveld's (1936) solution which was frequently replaced in order to avoid exposure to enzymes escaping from the digestive organs. A Lucite rod was then fixed to the mid-dorsal carapace so that the animal could be held suspended in Ringer in its normal primary orientation. Alternatively, the thorax was pinned in a wax dish which was shaped to allow the abdomen to move freely. Micromanipulated platinum wire hooks were used to stimulate the MG and LG axons after they had been dissected out of one of the connectives. The movements of the abdomen were recorded by photographing them at 1000 frames/s with a high-speed Hycam rotating-prism camera (Red Lake Labs., Sunnyvale, Ca.). The positions of the abdomen at chosen times during a tail-flip could be traced from single frames using a stop-frame projector equipped with a frame counter.

For studying the influence of giant-fibre impulses on muscles in the abdomen and in the appendages of the last segment, we pinned the animal ventral side up in a Lucite dissecting dish partially filled with paraffin, and exposed the abdominal nerve cord so as to leave the roots intact in each segment. This preparation is essentially identical to that described by Evoy & Kennedy (1967), except that to record fast flexor motor outflow we recorded from the transverse, anterior oblique, and posterior oblique muscles in the main abdominal segments with suction electrodes (for a description of these muscles, see Rayner & Wiersma, 1967). Various muscles of the uropods and telson (Larimer & Kennedy, 1969*a*) were exposed and recorded from in the same way, or dissected peripheral branches of the roots innervating them were attached to suction electrodes for recording impulses in specific motor axons. In all experiments of this sort, the giant fibres were isolated for stimulation through suction electrodes in a rostral abdominal connective.

Specific motor neurones leaving the sixth ganglion via its sixth root and innervating the telson flexor and anal compressor muscles were also studied anatomically in order to verify the physiological observation that they made differential synaptic contacts with LG and MG axons. The motor axons in this root were impaled with micropipettes filled with a saturated (*c.* 4%) aqueous solution of Procion Yellow M-4RAN (Imperial Chemical Industries America, Inc.). This fluorescent dye remains within cells and diffuses into even relatively fine processes, and is thus suitable for direct visualization of the ganglionic architecture of such neurones (Stretton & Kravitz, 1968; Remler *et al.* 1968). In our experiments it was injected by pressure; the preparation was then kept in the cold for about 12 h to insure sufficient time for diffusion into the ganglion. The sixth ganglion was then fixed in Lavodowsky's solution, dehydrated, and cleared in methyl salicylate. Whole mounts were made in Fluoromount and photographed in a Zeiss fluorescence microscope using excitation filter I and barrier filter 50. Such a preparation could later be resuspended in xylol to free it from the

mounting medium, embedded in paraffin, and sectioned at 10 μ. Complete reconstructions of the branching system were not made, but each cell was analysed to see which of the giant fibres its processes contacted. Similar methods were used to inject the giant fibres in the 5–6 connective, so as to visualize their terminations in the sixth ganglion.

RESULTS

1. *Cinematographic analysis of giant-fibre responses*

The movements of the abdomen in response to stimulation of LG and MG axon in the circumoesophageal connectives were photographed at 1000 frames/s; tracings of frames from such sequences are shown in Fig. 1. The differences between the two kinds of tail flexion are obvious and consistent. LG responses involve promotion of the exopodites of both uropods and a relatively incomplete flexion of the abdomen except at the junction of the thorax and in the last segment. Specifically, there is strong flexion of the anterior abdominal segments, but very little flexion of segment 4 in relation to 3 or 5 upon 4. The result is a 'sculling' stroke in which much of the thrust is vertical rather than horizontal. MG impulses, on the other hand, generate very complete flexion of all abdominal segments and the telson, but this movement is unaccompanied by flaring of the uropods. The stroke ends with the abdomen in a tightly curled position, and so produces a primarily horizontal thrust. Similar results have been obtained by Wine (personal communication), who also showed that LG flips in untethered animals actually produced the expected upward movement.

Flicks produced spontaneously or by natural stimulation were also photographed in the hope that the central pathway involved could be deduced by comparing them with those evoked by giant-fibre stimulation. Some movements, especially those stimulated by tapping in the abdominal region, resembled LG responses in that the flexion of intermediate segments was poorly developed and the exopodites of the uropods were promoted. Others were more complete, like MG flips, but differed from MG responses in the involvement of uropod appendages. We were left with the impression that pure responses of either kind rarely occurred spontaneously, and that most such movements probably involved pathways other than the giant fibres. At the time, we demonstrated that single fast flexions could be evoked by natural stimuli without giant-fibre involvement; and Schrameck (1970) has since shown that giant-fibre impulses are not usually involved in swimming.

2. *Electrical responses of abdominal muscles to giant-fibre activity*

The behavioural data suggested that the LG and MG axons made selective central connections with motor neurones innervating the muscles of the abdomen and of the tail appendages. We therefore recorded from individual muscles or the peripheral motor nerve branches innervating them while stimulating giant axons isolated from one of the central connectives.

Before presenting these results, we find it necessary to deal in a more general way with the junctions that connect the central giants and fast flexor motor neurones. Since it was first discovered that such a pathway exists (Wiersma, 1938, 1947), it has been shown to be a duplex one. A large cell, the motor giant, makes connections with both lateral and medial giant fibres in each abdominal segment. The connexions are

rectifying electrotonic junctions (Furshpan & Potter, 1959), and the motor giant cell innervates each fibre of the main flexor muscles of one side. The peripheral neuromuscular junctions, however, show rapid antifacilitation at low frequencies of stimulation, so that only the impulses in a well-rested preparation are capable of recruiting

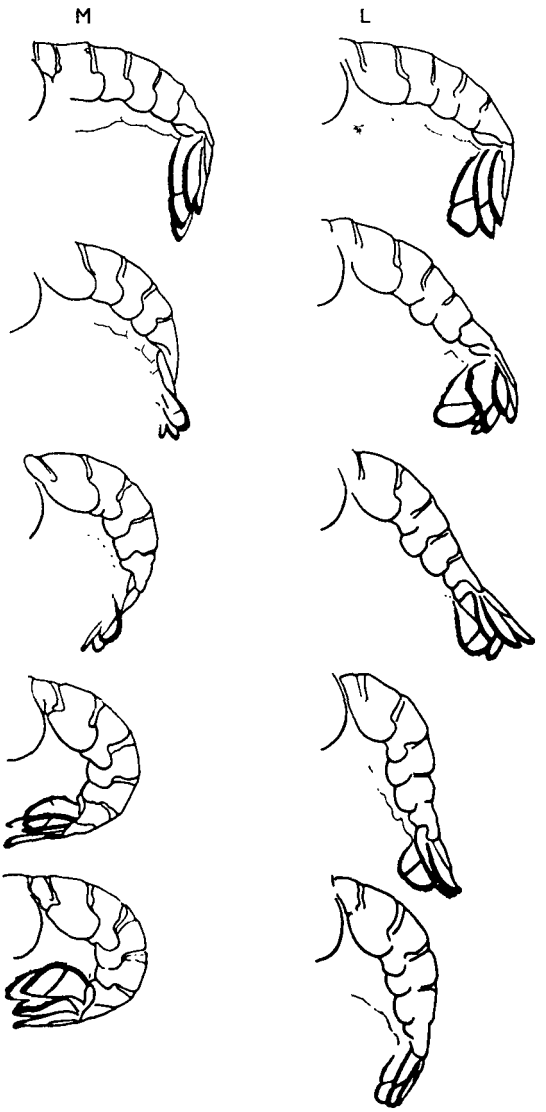

Fig. 1. Tracing from high-speed 16 mm ciné film of the abdominal movements of crayfish resulting from stimulating the isolated medial giant (M) and lateral giant (L) in the circumoesophageal connectives. The films were taken at 1000 frames/s; animals were held firmly by a rod attached to the thorax, but the abdomen was free to move. The tracings were made at 15 ms intervals.

substantial tension in the muscles (Kennedy & Takeda, 1965; Bruner & Kennedy, 1970). The eight other motor axons in each main abdominal flexor root innervate single muscle groups or parts of them, and maintain their efficacy even at fairly high

frequencies. The central junctions between the giant fibres and these motor neurones are located in the ganglia rather than at the root exit, where the motor giant contacts the central giants; they are formed by branches from the motor neurones that contact the axis cylinders of the central giants (Remler, Selverston & Kennedy, 1968). It is not yet known whether these are electrotonic or chemical synapses, but they exhibit a delay that is longer than that for the motor giant synapse.

One expects, then, that records from a third root in response to stimulation of a central giant axon will show two components: a short-latency discharge consisting of the single motor giant impulse, and a slightly delayed compound action potential

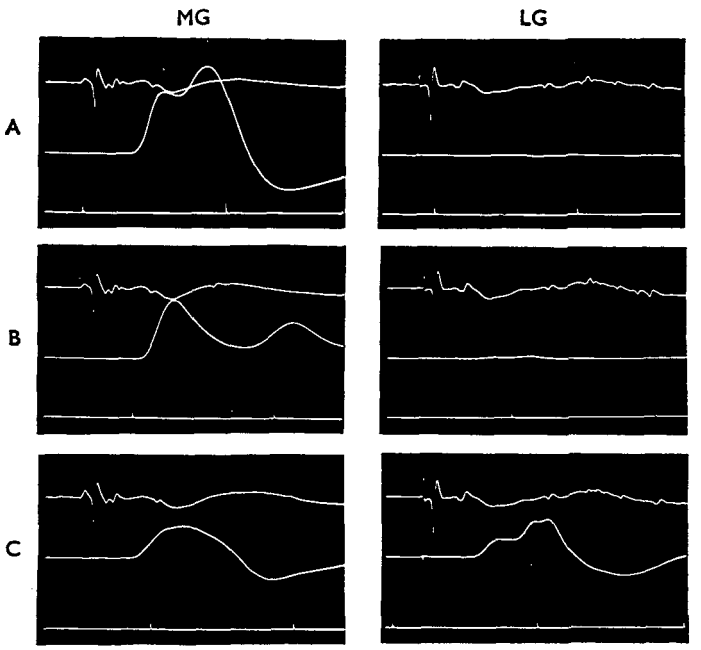

Fig. 2. Responses of the flexor muscles in the third abdominal segment following separate stimulation of MG and LG axons in the first abdominal connective. Upper trace, giant axon spike; lower trace, muscle responses; both were recorded extracellularly with suction electrodes. A, Anterior oblique muscle, following MG and LG stimulation: B, Posterior oblique; C, Transverse muscles. Only the MG axons were effective in driving all of the main flexor muscles of the abdomen. Time calibration, 10 ms.

composed of impulses in several of the 'non-giant' motor axons. Such records have, in fact, been published (Kennedy & Takeda, 1965), but the predicted output is often not obtained. The early records obtained from third roots by Wiersma (1947), for example, fail to exhibit two components, and most of them appear to consist of spikes from one to several 'non-giant' neurones. We have frequently failed to observe the motor giant response ourselves, and in fact Furshpan & Potter (1959) report that in the majority of their experiments on the LG-motor giant junction, it was not transmitting.

This perplexing unreliability also applies to the junctions between central giants and the non-giant motor neurones. The number of units responding in the main roots to activity in a given giant fibre is somewhat variable, as is the response of identified

motor neurones recorded in the periphery. For this reason a large number of experiments on each muscle or motor neurone was necessary to determine the presence or absence of a central connexion with the giant fibres. In none of the cases in which we report the absence of a connexion was one ever found; but some of the demonstrated connexions were not functional in some preparations. We do not know whether these inconsistencies demonstrate a real variation in the efficacy of junctions, or whether they are due to some experimental variable that we have been unable to control.

Figure 2 shows the responses of the major groups of phasic flexor muscles in the third abdominal segment to MG (left) and LG (right) impulses, following repetitive

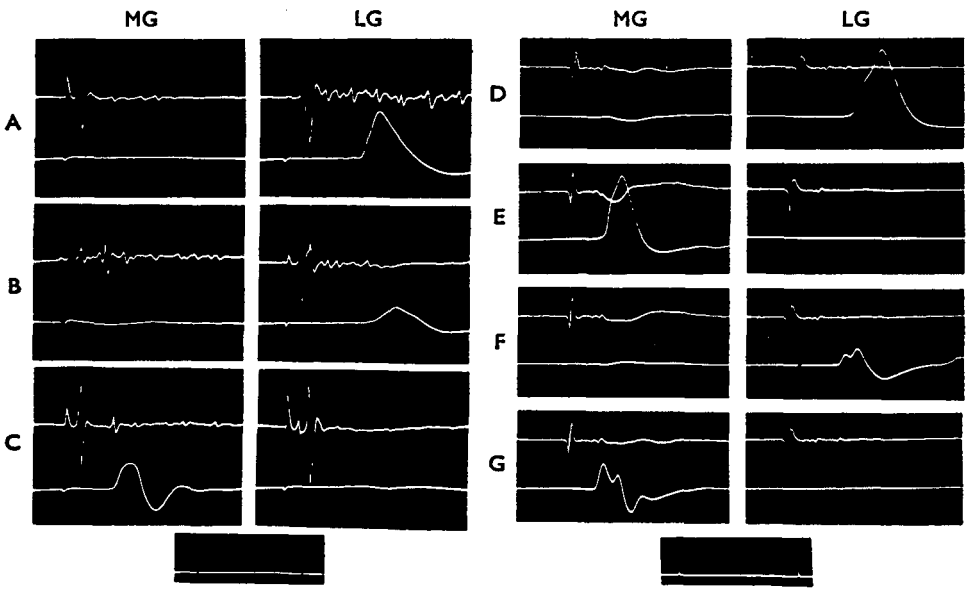

Fig. 3. Activity of muscles in the telson-uropod segment in response to electrical stimulation of MG and LG axons. Upper trace, giant axon spike; the lower trace, muscle potential; both were recorded externally with suction electrodes. A, Productor exopodite muscle; B, telson-uropodalis posterior; C, Posterior telson flexor; D, anterior telson flexor; E, anal compressor (ventral telson flexor); F, lateral remotor; and G, Ventral rotator muscle. Muscles involved in records A–F are innervated from the sixth ganglion; the ventral rotator is innervated from the fifth ganglion. Time calibration, 10 ms.

stimulation at low frequency. The MG strongly drive both the anterior and posterior oblique muscles as well as the transverse muscle, whereas the LG excite only the transverse muscle.

An even clearer differentiation of giant-mediated output occurs in the fifth and sixth ganglia (Fig. 3). Here, the MG axons drive the major flexors of the telson-uropod segment; i.e. the anal compressor (Fig. 3E; the suggested name given by Larimer & Kennedy, 1969*a*, for this muscle is the ventral telson flexor), the posterior telson flexor (Fig. 3C) and the ventral rotator muscle (Fig. 3G). The first two of these muscles are innervated from the sixth root of the sixth ganglion, while the latter is innervated via the third root of the fifth ganglion. All three of these muscles are probably homologous to the oblique series. In addition, however, the LG mediate contraction of three smaller phasic muscles in this region, viz. the productor exopodite of the uropods

(Fig. 3A), the telson uropodalis posterior muscle (Fig. 3B) and the lateral remotor muscle (Fig. 3F). None of these muscles receive motor neurones that are driven by both LG and MG axons.

3. *Excitation of motor neurones by giant-fibre activity*

The central giant fibres were found to drive motor neurones in the sixth ganglion whose axons emerge from the second, third and sixth roots. Because a characteristic branching pattern occurs in the sixth root, it was possible to identify the processes of individual motor neurones and determine whether they were activated by the giant fibres. Fig. 4 shows potentials obtained from various branches of the sixth root when the LG and MG axons were stimulated separately. LG were found to activate the

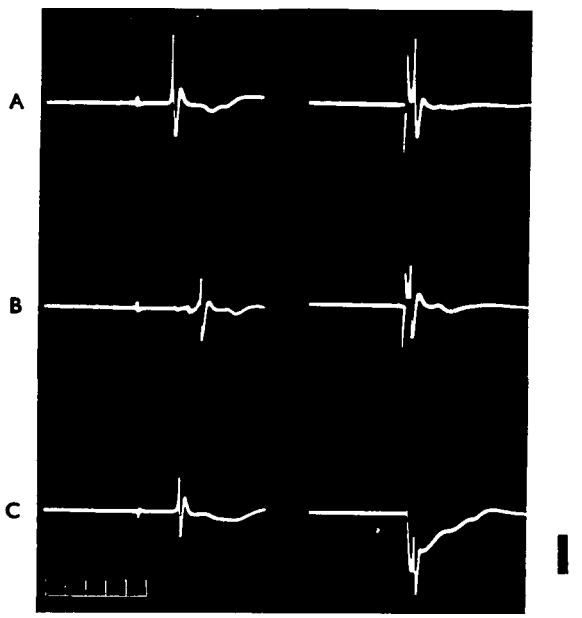

Fig. 4. Records of impulses obtained from the large axons in the branches of the sixth root of the last abdominal ganglion. The three traces on the left were obtained by giant-axon stimulation, those on the right from direct root stimulation. A. Impulses from the axon to the anterior telson flexor evoked by the LG axon (left) and by a direct root stimulus (right). B. Similar record for the large motor axon supplying the anal compressor (ventral telson flexor) in response to MG (left) and root stimulation (right). C. Response of the axon to the posterior telson flexor muscle to MG (left) and to root stimulation (right). Time calibration, 10 ms.

large motor axon of the anterior telson flexor (Fig. 4*a*, left). The same axon was identified (Fig. 4*a*, right) by stimulating the root directly and observing the corresponding contraction of the anterior telson flexor muscle. Fig. 4, *b* and *c*, shows the responses of motor neurones supplying the anal compressor (ventral telson flexor) and the posterior telson flexor to stimulation of the MG and the root directly. These data are consistent with those presented in Fig. 3 above, and additionally allow the identification of individual motor axons.

4. *Anatomical relations between giant fibres and motor neurones in the sixth ganglion*

Three large axons can be identified in the intact sixth root of the sixth abdominal ganglion. In order to visualize the cells directly, each was injected with Procion Yellow M-4RAN and fixed and cleared for fluorescence microscopy. Injections were usually carried out with the distal terminals intact. Under these conditions the dye penetrated distally to the innervated muscle, as well as centrally to the cell body in the ganglion. In order to visualize the terminations of the central giant fibres in the sixth ganglion, these axons were also injected with the dye and examined by fluorescence microscopy. The motor neurones to the anterior telson flexor, the ventral telson

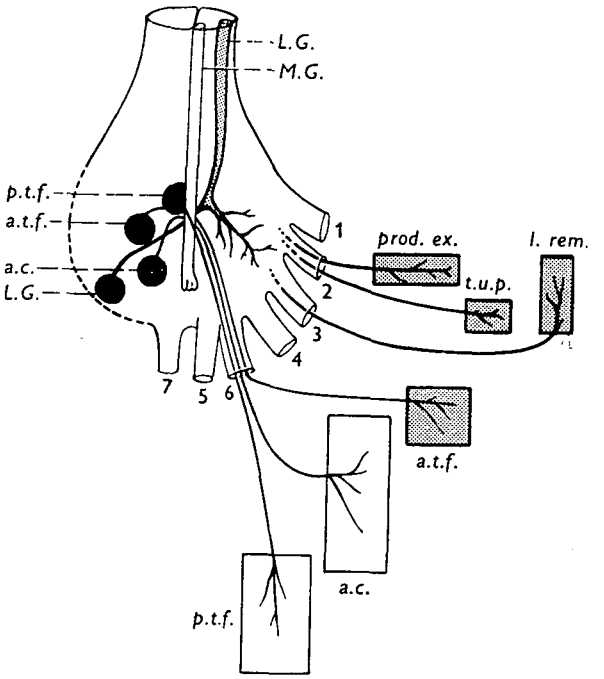

Fig. 5. Diagrammatic representation of the terminations of LG and MG axons in the sixth abdominal ganglion, and of the motor neurones and muscles that they affect. *L.G.*, lateral giant axon; *M.G.*, medial giant axon; *Prod. ex.*, productor exopodite; *l.rem.*, lateral remotor; *t.u.p.*, telson-uropodalis posterior; *a.t.f.*, anterior telson flexor; *a.c.*, anal compressor (ventral telson flexor); *p.t.f.*, posterior telson flexor.

flexor and the posterior telson flexor, as well as the terminations of the central giants in the sixth ganglion, resemble their presumed serial homologues in the third ganglion (Remler *et al.* 1968). The cell bodies are located contralaterally at the base of the third root near the posterior end of the ganglion. The somata communicate with the main axon via a very thin neurite while a large dendritic tree emerges from the junction of the neurite and the main axon. We have no evidence for the 'extra' LG segment in the sixth ganglion suggested by Johnson (1924). The MG axons end without major branches in the region of the base of the sixth root of the ganglion; short, knob-like endings are, however, generally present.

Some of the injected motor axons (Pl. 1) were sectioned at 10 μ and examined to confirm the morphology of the synaptic connections indicated in the electrophysiological experiments (Figs. 3, 4). As an example, contacts between the motor axon to the posterior telson flexor and MG is shown in Pl. 2. This axon enters the dorsal side of the ganglion through root 6. After coursing forward a short distance it becomes narrow, turns ventrally, passes near and sends out numerous small contacts that end on the MG. These contacts are assumed to be the functional synapses between the two neurones, since similar contacts are not made upon LG processes that lie nearby (cf. Kennedy, Selverston & Remler, 1969; Davis, 1970). Similar observations were made upon other identified motor neurones to the major telson muscles.

A summary diagram of the motor neurones, the giant fibres, and the muscles activated by the latter is given in Fig. 5. The LG axons supply excitation to four muscles in the terminal segments and appendages of the animal: the anterior telson flexor, the lateral remotor, the productor exopodite and the telson-uropodalis posterior. The MG axons activate the ventral telson flexor (anal compressor) and posterior telson flexor. LG axons of the two sides have a functional commissural synapse in the sixth as they do in the ganglia of more anterior segments, while the MG are not cross-connected.

DISCUSSION

The different central connections made by LG and MG neurones adequately explain the differences in motor behaviour observed when the central giant fibres are stimulated separately. A comparison of the movements commanded by MG activity (Fig. 1) with data on the muscles that are selectively activated by these interneurones (Figs. 2, 3) shows that the complete flexion of the abdomen is the result of a uniform activation of the oblique muscles of each anterior abdominal segment, with simultaneous excitation of the homologous flexor muscles of the telson-uropod segment (ventral rotator, and ventral and posterior telson flexors). The movements resulting from LG activity are somewhat more complex than those evoked by MG. They too, however, can be accounted for in terms of central connexions between the giant fibres and identified motor neurones. The incomplete abdominal flexion (Fig. 1) is probably the result of weak activation by the LG of motor neurones supplying oblique muscles in the central abdominal segments (Fig. 2); uropod promotion comes about through activity in motor neurones innervating the productor exopodite muscles.

The neural network involving motor neurones and central giant fibres in the terminal ganglion is more complete than that in more rostral segments of the abdomen. In the third ganglion, for example, all flexor motor axons are collected into the third root and all extensors into the second; whereas in the sixth ganglion phasic motor neurones of the several sorts emerge from roots 1, 2, 3 and 6. The MG axons synapse with the sixth root axons, while LG connect with motor neurones leaving roots 2 and 3 as well as 6. Since root 6 leaves the dorsal surface of the ganglion and contains most of the large phasic flexor motor axons, it is probably homologous with the third root of the anterior abdominal ganglia. The homologue of the motor giant neurone in the sixth ganglion, if present, would therefore be expected to send its axon out via the sixth root. We have, however, been unable to identify a neurone in the sixth ganglion that possesses the unique characteristics of the motor giants of anterior segments. For

example, none of the 6th root axons is driven by both MG and LG axons; furthermore, the 6th root contains three or four especially large axons instead of one, and all make *en passant* synapses with the giant fibres.

Several 'escape' behaviours that differ from one another in detail may be present in crayfish. High-speed photography of animals producing tail flips with their circumoesophageal connectives cut reveals that these movements differ from those obtained from intact animals. Even in the same intact animal spontaneous flips are not consistent in form from trial to trial. Since giant axons are not required for at least some of these responses (Schrameck, 1970), it is likely that several other interneurones or combinations of them, in addition to the central giants, can synchronously activate the fast flexor muscles. For the phasic extensors of the abdomen and several other muscles—including the rotators, remotors and adductors of the telson-uropod region (Larimer & Kennedy, 1969*a*)—the normal pre-motor elements are unknown. Certain specific responses, such as swimming or steering, may incorporate these additional motor elements only under very occasional and specific commands.

The demonstration that specific interneurones (the giant fibres and perhaps others) can activate ensembles of phasic motor neurones in different combinations is of interest because it is now clear (Larimer & Kennedy, 1969*b*) that a comparable system of interneurones exists for the tonic system of uropod muscles. Such a group of interneurones acting upon the phasic elements could provide for a wide range of rapid, stereotyped movements comparable to those known to be available in the postural system.

SUMMARY

1. High-speed cinematography was used to analyse the abdominal movements of crayfish in response to separate stimulation of medial and lateral giant axons. These films showed that the medial giant fibres command complete abdominal flexions with little flaring of the tail appendages. The lateral giants, in contrast, evoked a relatively weak flexion of the middle abdominal segments, accompanied by promotion of the exopodites of the uropods.

2. An examination of the muscles activated by the two types of giant fibres shows that differences in the connexions between the giant fibres and specific motor neurones can account for the behavioural differences observed.

3. The output of the giant fibres was determined in the sixth abdominal ganglion, where their differential effects are most pronounced. The medial giants activate motor neurones whose axons emerge from root 6 of the sixth ganglion. The lateral giants activate motor neurones whose axons emerge via roots 2 and 3, as well as those emerging via root 6.

4. The larger motor neurones associated with the giant axons in the sixth root of the sixth ganglion have been mapped by Procion Yellow injection, and the terminations of the central giant axons in the sixth ganglion have also been determined. The connexions revealed by this technique are consistent with the physiological findings.

5. The evidence suggests that root 6 of the sixth ganglion is homologous with root 3 of the more anterior ganglia. However, the giant motor neurone of the sixth ganglion has not been identified.

6. The medial and lateral giant fibres, and perhaps other specific 'command'

interneurones, can thus drive specific ensembles of phasic motor neurones to provide a range of stereotyped quick movements. In this respect the organization of the phasic system of interneurones and motor neurones resembles that in the tonic system.

The experiments on cinematography and on the influence of LG and MG stimulation on abdominal and uropod muscles were carried out at Stanford during 1967–68. This work was supported by grants from the U.S. Public Health Service (NB-02944) and the Air Force Office of Scientific Research (AFOSR-68-1373) to D. Kennedy and by a Guggenheim Fellowship award to J. Larimer. The experiments on identified sixth-ganglion motor neurones and the dye injection studies were carried out at Texas during 1969 and 1970, and were supported by a grant to J. Larimer from the U.S. Public Health Service (NS-05423) and by The NIH Biomedical Sciences Support Grant 5-S05 FR-07091-04. L. M. Masukawa and A. C. Eggleston were supported by NIH Training grant 2 T01 GM 00836-07. We acknowledge the unfailing technical assistance of Joanna Hanawalt and Gary Shelton, and thank J. Schrameck and the late Dr D. M. Wilson for helpful discussions and a critical reading of the manuscript.

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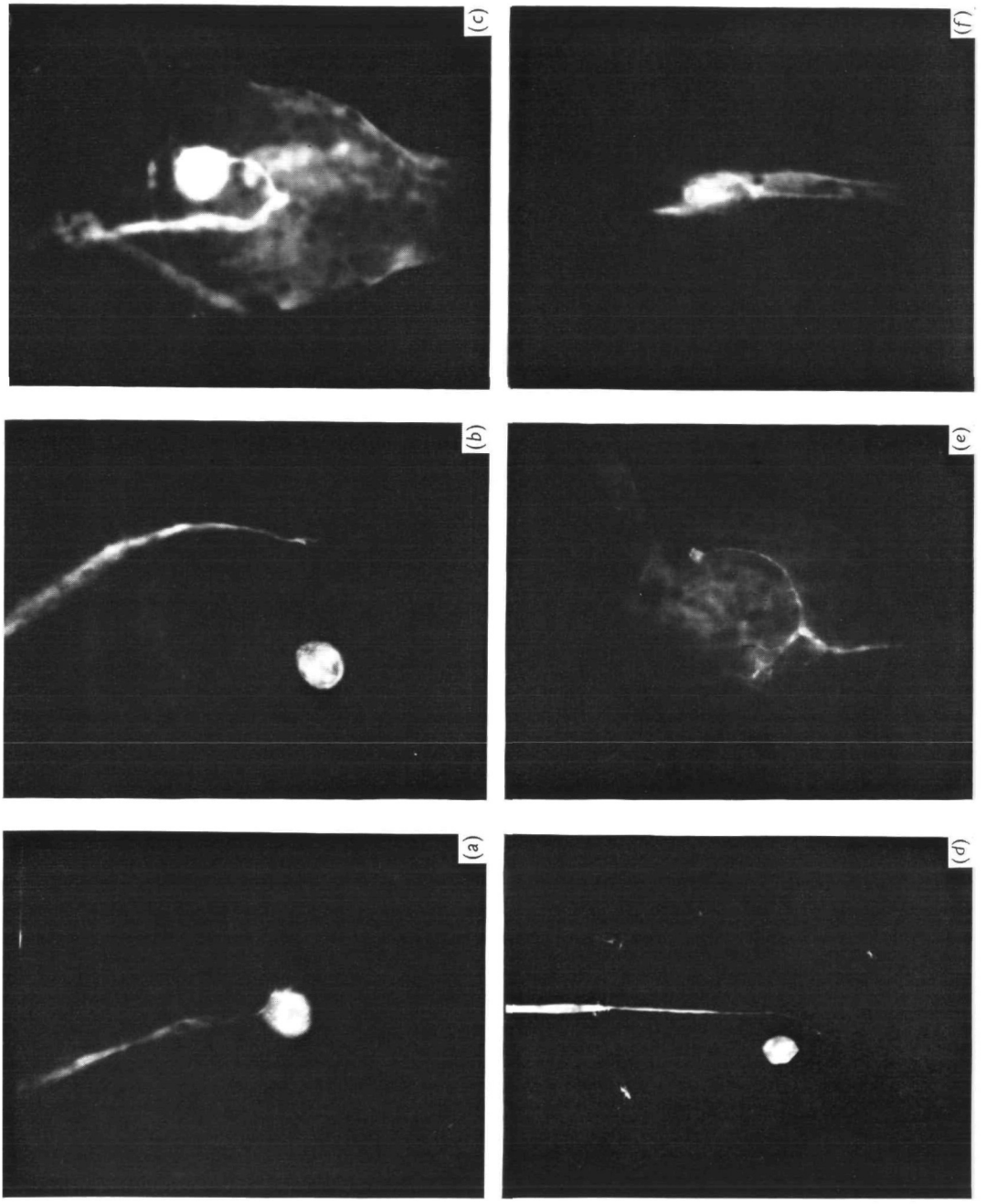

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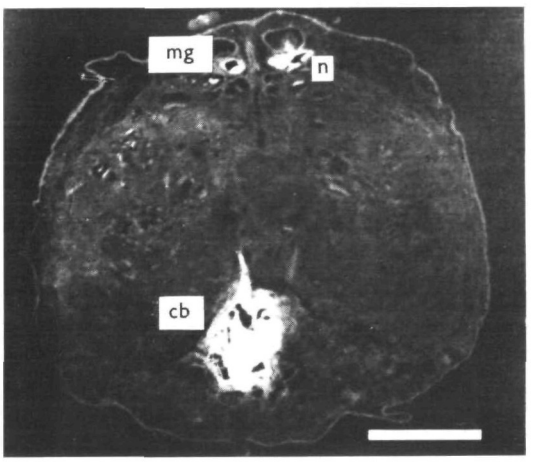
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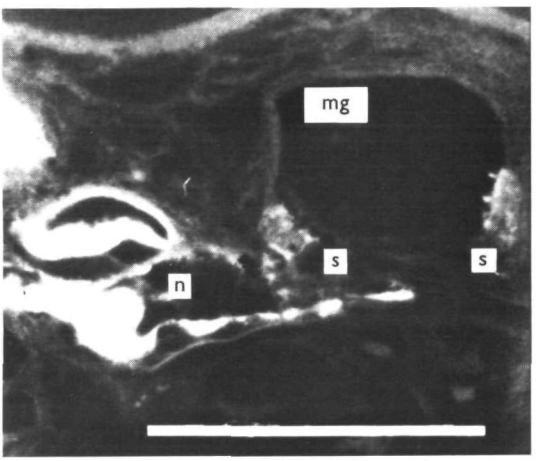
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EXPLANATION OF PLATES

PLATE 1

Fluorescence photomicrographs of Procion Yellow-injected giant fibres and motor neurones in whole mounts of the sixth ganglion. (*a*) Upper left: MG axon. There is some leakage of dye into one of the large motor neurones of the sixth root, probably at the junctions. (*b*) LG. (*c*) The large motor neurone supplying the anterior telson flexor (retouched). (*d*) The motor neurone supplying the anal compressor muscle (ventral telson flexor muscle). (*e*) Side view of the large motor neurone to the posterior telson flexor. (*f*) The same p.t.f. neurone as seen from the ventral side of the ganglion. Injections of this type allowed the placement of the neurones in the map of Fig. 5. Scale: 100 μ.

PLATE 2

Photomicrographs of cross-sections of the sixth abdominal ganglion, showing a Procion Yellow-injected posterior telson flexor motor neurone cell body (*cb*) and neurite (*n*) with synapses (*s*) on MG. Scale left and right: 200 μ, 50 μ.