J. exp. Biol. **127**, 279–294 (1987)
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THE TELSON FLEXOR NEUROMUSCULAR SYSTEM OF THE CRAYFISH

II. SEGMENT-SPECIFIC DIFFERENCES IN CONNECTIVITY BETWEEN PREMOTOR NEURONES AND THE MOTOR GIANTS

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Accepted 26 June 1986

SUMMARY

1. The telson and sixth ganglion of the crayfish contain a fast flexor system that is homologous to that found in anterior segments, but doubled (Dumont & Wine, 1986*a*). In this paper we document differences in connections to the motor giants (MoGs) in the telson as compared to the MoGs in the anterior five abdominal segments.

2. Unlike their homologues in anterior segments, the telson MoGs receive excitatory input *via* a trisynaptic pathway that is activated by the escape command axons, the lateral and medial giants (LGs and MGs), and includes the identified corollary discharge interneurones I2 and I3. For I3, at least, the connection to the MoGs is monosynaptic, electrical and rectifying, and is sufficiently strong that simultaneous activation of the two I3s alone fires the telson MoGs.

3. The trisynaptic pathway from the LGs to the telson MoGs is inhibited by central, command-derived, postsynaptic inhibition of the telson MoGs, which typically arrives earlier than the excitation. In experimental preparations, this inhibition can be partially circumvented by stimulating the LGs anywhere anterior to the third abdominal ganglion. This is possible because the polysynaptic excitatory pathway is recruited in the third ganglion, while inhibition is recruited by the LGs locally in the sixth ganglion. Hence the site of impulse initiation in the LG affects the relative timing of excitation and inhibition of the telson MoGs. This arrangement makes it possible, in principle, for the site of impulse initiation in the LG to affect the form of the resulting tailflip.

4. In dissected preparations, LG impulses initiated anterior to the third ganglion fired the telson MoGs in 16 out of 25 experiments, while impulses initiated posteriorly never fired the telson MoGs (nine experiments).

5. Behavioural studies indicate that anterior stimuli which evoke LG activity do not cause activation of the telson MoGs. We suggest that in intact animals inhibition of the telson MoGs is more effective than in physiological preparations.

6. As far as we can tell from available evidence, the I3 input to the telson MoG is never expressed, and therefore cannot be explained in functional terms. We

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Key words: escape behaviour, homology, invertebrate, motor patterns.

suggest that the differences between the inputs to the MoGs of the telson and of the fourth and fifth ganglia is the incidental result of developmental constraints during evolution.

INTRODUCTION

In the previous paper (Dumont & Wine, 1986*a*), we identified components of the telson flexor neuromuscular system and established homologies between them and the fast flexor system. In this and the following paper, we describe differences between segmentally homologous cells and attempt to determine reasons for the differences. We begin with the telson motor giants (MoGs) which are especially well suited for our purposes. Among their advantages is their unambiguous homology with MoGs of anterior segments, which have limited and well-described inputs and are important components in a discrete set of stereotyped behaviours. These features have helped restrict our interpretations, and provide a point of departure for the analysis of the other efferents (Dumont & Wine, 1986*b*).

The purpose of this comparative study is to try to understand how neural circuits evolve. The logic behind this is as follows. As these segmentally repeated circuits are homologous, they were once identical, or at least a great deal more similar than they are now. This assumption is based on comparative and developmental evidence (Keyser & Lent, 1977; Lawrence & Morata, 1983; Loer, Steeves & Goodman, 1983; Schram, 1982). Therefore the differences that we see today have arisen as a result of divergent evolution. Analysis of these differences should therefore enable us to investigate the events that generated them.

This has been done with some success in a number of other studies, most of which relate differences in circuitry to important functional specializations, implying a dominant role for selection in their evolution. For example, the motor neurones of the locust jumping leg are interconnected by a network of mutual excitation (Heitler & Burrows, 1977), whereas the homologous neurones innervating the walking legs are not (Wilson & Hoyle, 1978), and this mutual excitation is an adaptation to allow the build up of tension necessary for jumping. More pertinently to the present paper, the caudal MoGs of the crayfish abdomen lack the synapse with the LGs that is found in the rostral MoGs, and this is essential for the shaping of the LG-mediated tailflip.

However, selection is not the only process guiding evolution; for example, random events and developmental constraints play a role (Gould & Lewontin, 1979; Gould & Vrba, 1982; Lewontin, 1978) and, at least in one example, the effects of this are seen. The sensory neurones innervating the skin of the leech are virtually identical in all midbody segments (Keyser & Lent, 1977) but they have smaller receptive fields in the head segments (Yau, 1976). This might be ascribed to the need for greater sensory acuity in the head, except that in this region the segments are themselves smaller. In fact, the sensory fields of these neurones are larger relative to segment size than elsewhere in the body, raising the possibility that the absolute decrease in area innervated occurred secondarily to the decrease in segment size. The comparative

study of the MoGs offers an opportunity to investigate the roles of adaptive and non-adaptive processes in the generation of segmental differentiation.

MATERIALS AND METHODS

The neuronal recording methods used were the same as those described in the previous paper (Dumont & Wine, 1986*a*). Although most of the experiments in this paper used the isolated nerve cord rather than the semi-intact preparation, for better resolution of the components of the PSPs, the results of these experiments were confirmed in both preparations.

Activation of corollary discharge interneurones (CDIs)

The I3s are fired indirectly by the LGs and MGs *via* the segmental giant interneurone in ganglion 3 (SG3, Fig. 1). Each SG has an axon in nerve 1 of its ganglion (Roberts *et al.* 1982) except the SG6 axon which exits *via* nerve 2 in G6 (Dumont & Wine, 1986*a*). Thus, by stimulating N1 G3 we could antidromically activate SG3, and hence the ipsilateral I3.

Behavioural experiments

Animals (6–8 cm) were stimulated with electrodes implanted in the exoskeleton, with electrodes implanted next to the nerve cord or manually with a rod. The stimulating electrodes were two twisted, insulated silver wires (76 μm diameter). For the exoskeletal electrodes, 0·5–1 mm insulation was removed from the tips of the wires and they were inserted through small holes punched through the exoskeleton 1–2 mm apart; the wires were held in place with cyanoacrylate adhesive. Such electrodes were placed in the cephalothorax, just posterior to the lateral suture about one-third of the way down the animal's side. Other electrodes were placed close to the lateral ventral edges of the second and fifth abdominal segments. Stimulus durations of 0·5–5 ms were used.

For stimulating the cord, a small patch of insulation was removed from the wires about 1 cm from the tips. A hypodermic needle was passed through the abdomen, piercing the two sides of one segment and passing over the dorsal side of the nerve cord without injuring the fast flexor muscles. The electrodes were threaded through the needle and the needle was then withdrawn. The position of the electrodes was adjusted to bring the bare patches over the nerve cord, and the wires were fixed on both sides of the animal with cyanoacrylate adhesive. Electrodes were implanted in segments 2 and 5. Stimulus durations of 0·1–1 ms were used.

The rod we used for tactile stimulation had a phonograph pick-up cartridge attached to the handle, with the needle touching the rod. The output from the cartridge was amplified and triggered a pulse generator (Ortec), which in turn triggered a strobe light at 20- to 30-ms intervals. When electrical stimulation was used, the stimulus and strobe (see below) were triggered simultaneously.

For photographing the behavioural response, a crayfish was placed in an aquarium with a matt black background in a dark room. (For the manual stimulation, a white

sheet of paper was placed under the aquarium and the crayfish was illuminated with a dim red light.) With the camera shutter held open, the animal was stimulated and the strobe triggered, so a sequence of 4–6 images was superimposed on one exposure. The trajectory of the tailflip was then reconstructed from the negatives.

To record the EMG from the posterior telson flexor (PTF) muscles, electrodes like those for exoskeletal stimulation were used. The electrodes were inserted through the dorsal surface of the telson. One lead was implanted into the PTF muscle and the other was inserted just medial to it.

All crayfish were immersed in ice for 30 min prior to inserting electrodes and recovered 24 h before experiments. Experimental trials were separated by 15 min.

RESULTS

Connections to the telson motor giants

Monosynaptic input

In thoracic ganglia 1–3 (Crabtree, 1981) and abdominal ganglia 1–3 (G1–G3) (Mittenthal & Wine, 1973), the MoGs are activated by a single impulse in either the MGs or the LGs. In abdominal ganglia G4 and G5, the MoGs receive no excitation from the LGs but are still fired by the MGs (Mittenthal & Wine, 1973). In G6 the pattern of monosynaptic input from the giant interneurones is the same as that found in G4 and G5: suprathreshold for the MGs and absent for the LGs (Dumont & Wine, 1986*a*). This pattern of connections is the main basis for the different type of tailflip produced by the LG compared to the MG (Fig. 1); it explains why the posterior abdomen and telson are flexed for MG-mediated tailflips, but not for LG-mediated ones.

Disynaptic input

In anterior ganglia, the SG does not synapse with the MoGs. This is not surprising since the dendritic zone of the MoGs is in the connective, far from the processes of the SG. In G6 the SG and MoGs both have processes within the ganglionic neuropile, yet we saw no electrophysiological evidence for contacts between them (Fig. 4C).

Trisynaptic input

Corollary discharge interneurones (CDIs) are intersegmental interneurones which are fired by the giant escape command axons (Kramer, Krasne & Wine, 1981*b*). They have diverse functions, and represent a heterogeneous population of intersegmental interneurones which are presently defined exclusively by their common input. Two prominent CDIs have been identified. They are called I2 and I3 according to the ganglia in which they originate; they are fired by the SGs in their ganglion of origin and were previously shown to produce small EPSPs in axial fast flexor (FF) motor neurones in G4 and G5 and large EPSPs in unidentified telson flexor motor neurones (Kramer *et al.* 1981*b*).

We have found that I2 and I3 produce large EPSPs in the telson MoGs, but not in their anterior homologues. The input from I3 is particularly powerful (mean = 15 mV recorded in the neuropile, $N = 7$). The response of the telson MoGs to activation of the giant interneurones is shown in Fig. 2. The excitatory inputs can be correlated with the time of arrival of the I2 and I3 spikes in G6 (recorded extracellularly from the connectives as they enter the ganglion). In G5 the MoGs do not receive this input. While the input from the I3s appears to be a single EPSP, it is in fact double, since both I3s are recruited by the giant command axons and each I3 projects to both sides of the ganglion and synapses on both telson MoGs. The EPSP elicited by activating a single I3 is shown in Fig. 2Biii.

The EPSP evoked by the I3s was often subthreshold (Fig. 2B). However, it can activate the MoGs under certain experimental conditions. To demonstrate this, we

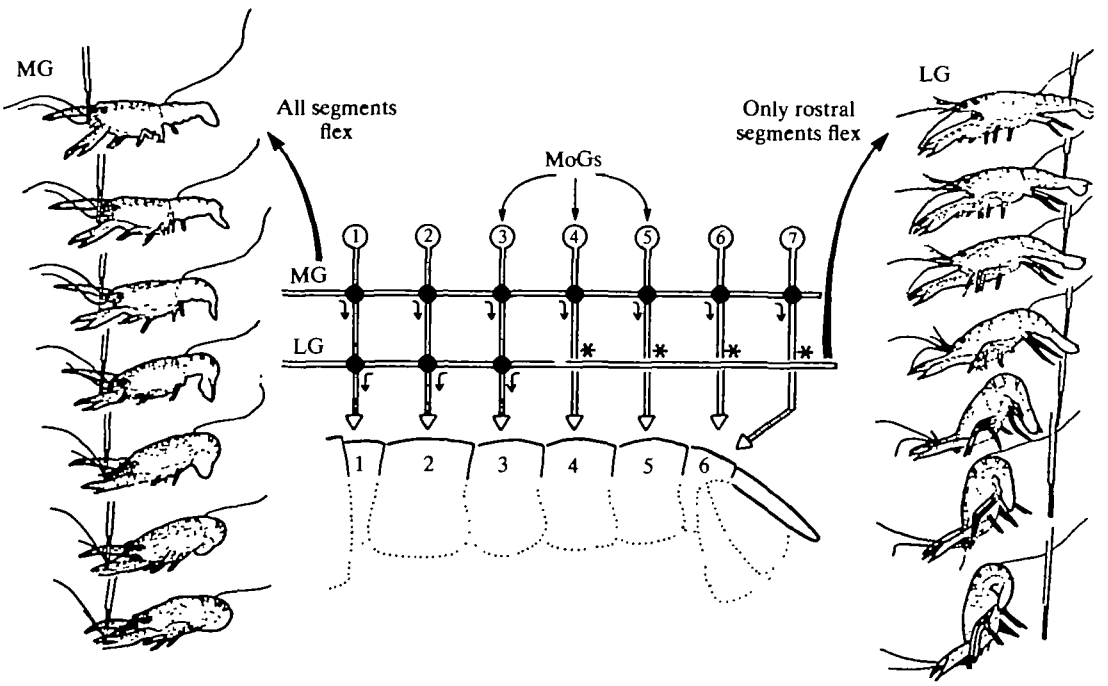

Fig. 1. Connectivity pattern of the giant command axons with the motor giants (MoGs) explains the orientation of the initial escape movement. The side panels are tracings from high-speed films that show the responses to tapping the head, which fires the medial giant (MG) command axons, or to tapping the abdomen, which fires the lateral giant (LG) command axons. When MGs fire, all MoGs are excited, all segments flex, and the abdomen curls and propels the animal backward. When the LGs fire, there is no output to the caudal segments, which remain straight and so cause the thrust to be directed mainly down, thus the animal pitches forward. Since the MGs respond to rostral inputs and the LGs to caudal ones, the tailflips remove the animal from the stimulus source. Approximately 50 ms of escape behaviour are shown. The centre panel is a scheme of the synaptic connections between the command axons (horizontal lines) and the MoGs (vertical lines). Direct, rectifying, electrical, one-to-one synapses are represented as filled circles; omitted synapses are indicated by asterisks. (Based on Furshpan & Potter, 1959; Wine & Krasne, 1972; Mittenthal & Wine, 1973, 1978; Dumont & Wine, 1986*a*.)

recorded from a telson MoG in an isolated nerve cord. We then activated the I3s independently of the rest of the command circuitry by stimulating the SGs in G3 (see Materials and Methods). In one experiment in which the I3s were activated in this way, a single I3 produced a 25 mV EPSP, and when both I3s were fired together the telson MoG was fired (Fig. 3A–C). In another experiment the telson MoG was monitored by recording its action potential in nerve 6 (it is recognizable by its large amplitude, rapid conduction speed and characteristic effect on the muscle; Dumont & Wine, 1986*a*). This procedure, which eliminated the possibility of altering the threshold of the telson MoG by insertion of a microelectrode, confirmed that the two I3s alone can fire the telson MoG; in one out of seven experiments a single I3 EPSP was sufficient to activate the telson MoG.

The I3-to-MoG synapse is electrical and shows the same properties of rectification (Fig. 3D–F) that were discovered in the giant-interneurone-to-MoG synapse (Furshpan & Potter, 1959). A similar synapse has been described linking the giant interneurones to the SG (Roberts *et al.* 1982), so this type of synapse appears to be fairly widespread within the system. The I2-to-MoG synapse appears to be similar, but its properties have not yet been investigated in detail.

Command-evoked inhibition of the telson MoGs

In all more anterior abdominal ganglia, activation of either pair of giant interneurones also produces a polysynaptic, multicomponent, long-duration, depolarizing IPSP which starts about 1–2 ms after the arrival of the impulse and lasts longer than 100 ms. A major component of this IPSP can be produced by stimulating any fast flexor motor nerve, or by stimulating the axons of one or more motor giant inhibitors (MoGIs) that run the length of the abdominal nerve cord and are excited in each ganglion except the last (Wine, 1977). As their name implies, the MoGIs appear to inhibit the MoGs exclusively (Wine, 1977). The telson MoGs also receive inhibition *via* this highly selective inhibitory pathway (Fig. 4B; Dumont & Wine, 1986*a*).

In addition to the interganglionic pathway, the telson MoGs also receive command-evoked inhibition *via* local interneurones in G6 (Fig. 4D). This was demonstrated by comparing the latency of the IPSP due to LG stimulation at different distances from G6. We found that the early components of the IPSP started with a constant, short latency from the LG spike (recorded in G6) regardless of where the LG was activated (Fig. 4D). In contrast, the delayed EPSP derived from I3 showed a position-dependent change in time of arrival since it is recruited in G3. One of these local MoG inhibitors has been identified (Kirk, Dumont & Wine, 1986). This cell is activated by SG6. In our experiments, SG6 is activated by antidromic stimulation (see Fig. 4C); it is activated naturally by MG or LG.

The effects of these command-evoked IPSPs are to reduce the possibility of the I3s firing the telson MoGs during a giant-induced tailflip. The possibility of this occurring during an MG tailflip are further reduced because the I3 input arrives during the refractory period after MoG activation by the MGs. As as result, I3s never activated MoGs during MG tailflips.

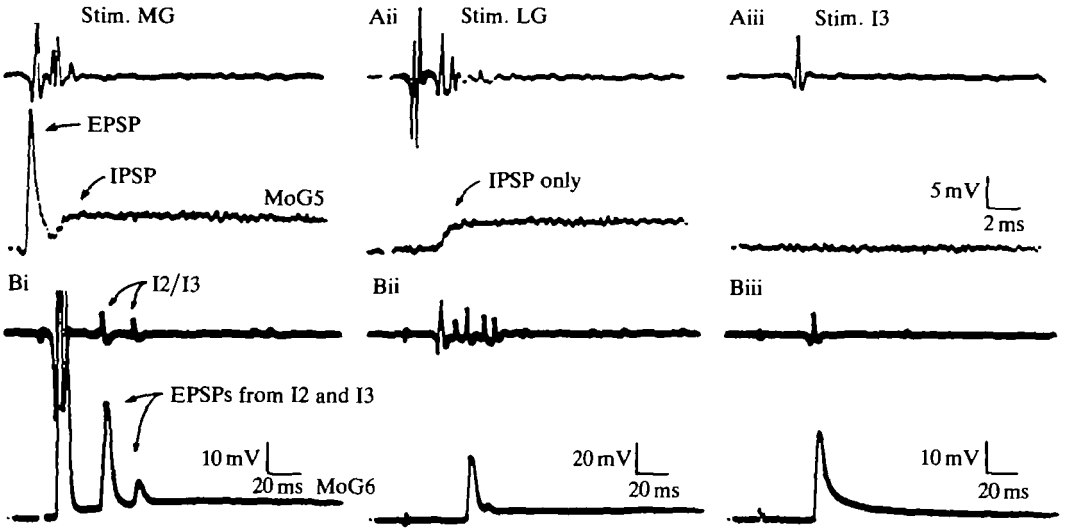

Fig. 2. Command-derived input to motor giants (MoGs) in G5 and G6. Neuropile recordings of MoG5 and MoG6, together with recordings of connectives. (A) MoG5 receives a single, suprathreshold EPSP from the medial giants (MGs), followed by an IPSP; the lateral giant (LG) produces only the IPSP; I3 has no effect. (B) MoG6 receives the same inputs as above, but also receives EPSPs from I2 and I3. These can be detected as components of the giant-evoked PSP, or by individual stimulation of I3.

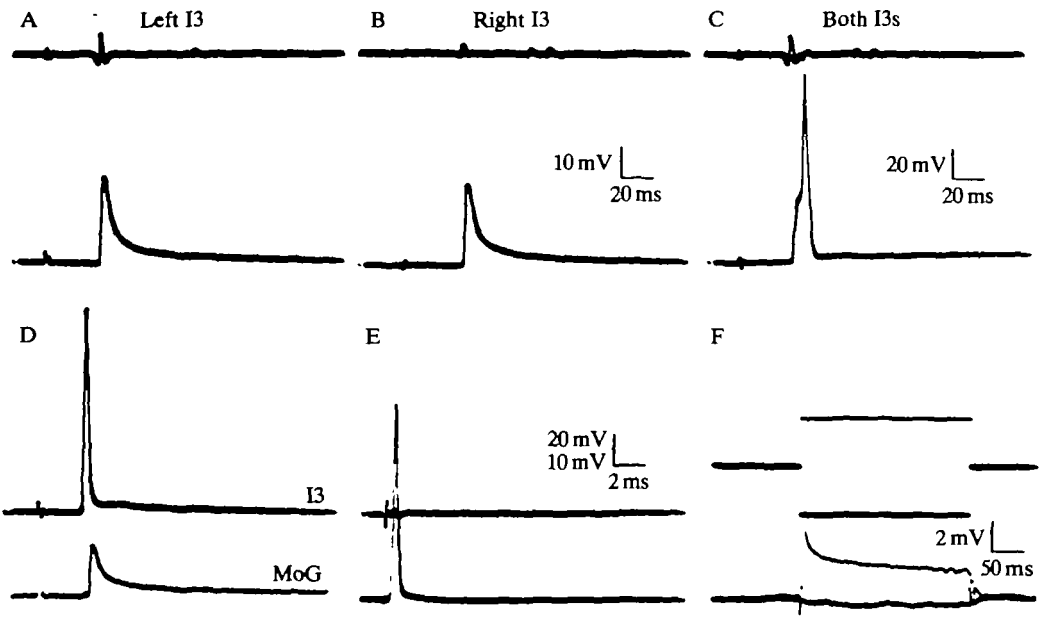

Fig. 3. Properties of the I3-to-MoG (motor giant) synapse. (A,B) Inputs from ipsilateral and contralateral I3s are almost identical. (C) Stimulation of both I3s together produces a suprathreshold EPSP. (D–F) The I3-to-MoG synapse is electrical and rectifying. (D) An action potential in I3 (top trace) produces a rapid, short-latency EPSP in MoG (bottom trace). (E) An antidromic spike in MoG has no effect on I3. (F) Current injection into I3 alone produces a change in MoG membrane potential only if it is depolarizing. (See Dumont & Wine, 1986*a*, fig. 3, for details.)

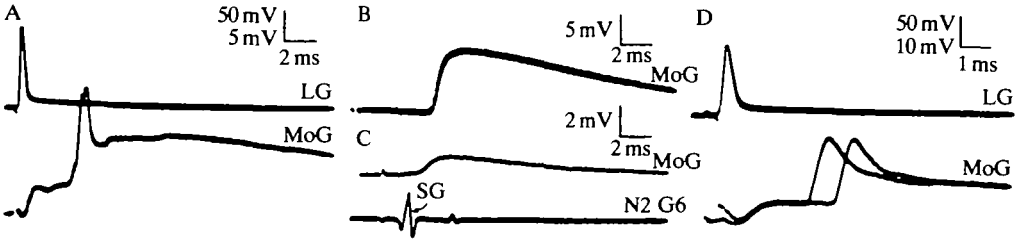

Fig. 4. Command-evoked inhibition of the G6 motor giants (MoGs) (all recordings from neuropile). (A) Compound PSP resulting from lateral giant (LG) stimulation. (B) Largest single identified component of command-evoked IPSP is from the motor giant inhibitor (MoGI), shown here. The MoGI was stimulated disynaptically by activation of fast flexor motor neurones in N3 G5 (see Wine, 1977). (C) Locally induced IPSP evoked by antidromic stimulation of segmental giant (SG). (D) Earliest components of IPSP are locally recruited. The LG and MoG were recorded in G6. The LG was stimulated in the connective at two sites: anterior to G5 and close to G6. The relative timing of the two stimuli was adjusted so that the intracellular LG spikes recorded in G6 were superimposed. Because the IPSP in MoG is locally recruited, its timing depends only on the time of arrival of the LG spike. In contrast, the EPSP from I3 is not recruited locally, and therefore shows a position-dependent change in latency.

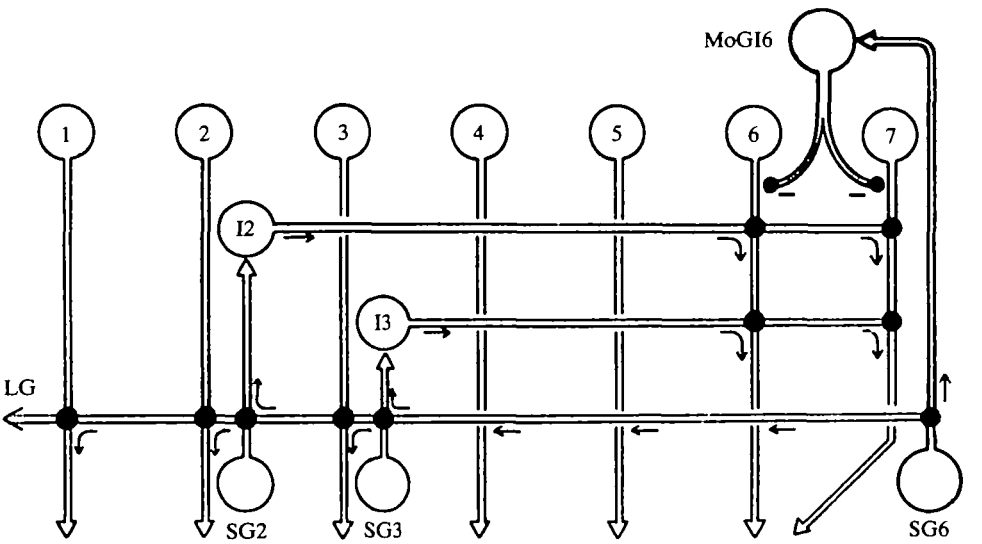

Fig. 5. Interaction of inhibition and excitation in telson motor giants (MoGs) when the lateral giant (LG) is fired. The LG activates the segmental giants (SGs) in all abdominal ganglia. In G2 and G3 these in turn fire I2 and I3, which produce EPSPs in the telson MoGs. However, in G6 local inhibitory interneurones are also recruited, and these shunt the input from I2 and I3.

Interaction between inhibition and excitation

When a potentially suprathreshold input is found to be inhibited in this fashion, it may be that it is effective under certain circumstances. One such circumstance is suggested by the rostral origin of the trisynaptic input to the telson MoGs. LG evoked inhibition of the telson MoGs is recruited locally, whereas I3 input

recruited in G3 (Fig. 4D). The relative timing of IPSP and EPSP therefore depends on where the LG is activated (see Fig. 5).

To test this possibility, an MoG6 was recorded in the neuropile of an isolated nerve cord, and the timing of the LG and I3 action potentials was monitored by recording extracellularly from the connective as it enters G6. The LG was then stimulated in different segments of the nerve cord, which resulted in different relative timing of the LG and I3 inputs. If the LG was activated in G5 (Fig. 6C), the action potential propagated in both directions. After a short delay it reached G6 and recruited the local inhibitors. The action potential also propagated forward and, slightly after it reached G6, activated the I3s in G3. There was then a further delay as the I3 impulses travelled to G6. By the time they produced an EPSP in MoG6, the IPSP was close to maximum and the EPSP was shunted. If the LG was stimulated at G4 (Fig. 6B), the delay in the arrival of the LG impulse was greater, and the I3 impulses arrived sooner. Hence the EPSP was less severely shunted and its amplitude was greater. If the LG was activated at G3 (Fig. 6A), the time lag between the arrival of the LG and I3 impulses was reduced to a minimum, and was due solely to the delay in activation of the I3s by the LGs and the difference in conduction velocity between the LGs and the I3s.

Our key finding was that when the I3 EPSP was minimally shunted, it was frequently suprathreshold, causing the telson MoG to fire (Fig. 6A) and the muscle to twitch. Firing the LGs anywhere anterior to G3 had the same effect. This experiment was repeated 25 times (Table 1). In 16 of these (64%), anterior LG activation fired the telson MoGs. Of those, nine were also tested to see if posterior LG activation was effective; it never was. In two of the semi-intact preparations, we also used electrical stimulation of sensory nerves in G2 or G3 to activate the LGs in order to test for a role of sensory input. In both cases the telson MoGs were activated. In all the minimally dissected preparations, the thoracic–abdominal connectives were intact. Also, in the minimally dissected preparations and some of the other preparations, the telson MoGs were recorded extracellularly. This had no effect on the probability of activation.

These results imply that activation of the LGs in abdominal segments 1–3 will often produce telson flexion, a component of the tailflip normally associated with the MG-mediated response. This would presumably cause the crayfish to escape with a more rearward trajectory than a standard LG-evoked tailflip, which might well be adaptive for these more anteriorly directed stimuli (see below).

Preliminary discussion

In previous papers, Kramer *et al.* (1981*b*; see their fig. 19) and Kramer, Krasne & Bellman (1981*a*) described a very similar pattern of synaptic inputs to a cell identified merely as a ventral telson flexor (VTF) motor neurone. It will be shown later (Dumont & Wine, 1986*b*) that telson FF motor neurones are also excited by I3, but are not centrally inhibited. Therefore, the unidentified VTF motor neurone discussed at length by Kramer *et al.* (1981*a*,*b*) was almost certainly the cell we have

labelled MoG7. Our results are completely consistent with the previous work, but we have identified the motor neurones as the telson MoGs, and have identified the sources of inhibition. These identities have implications for interpreting the significance of the connections (see Discussion).

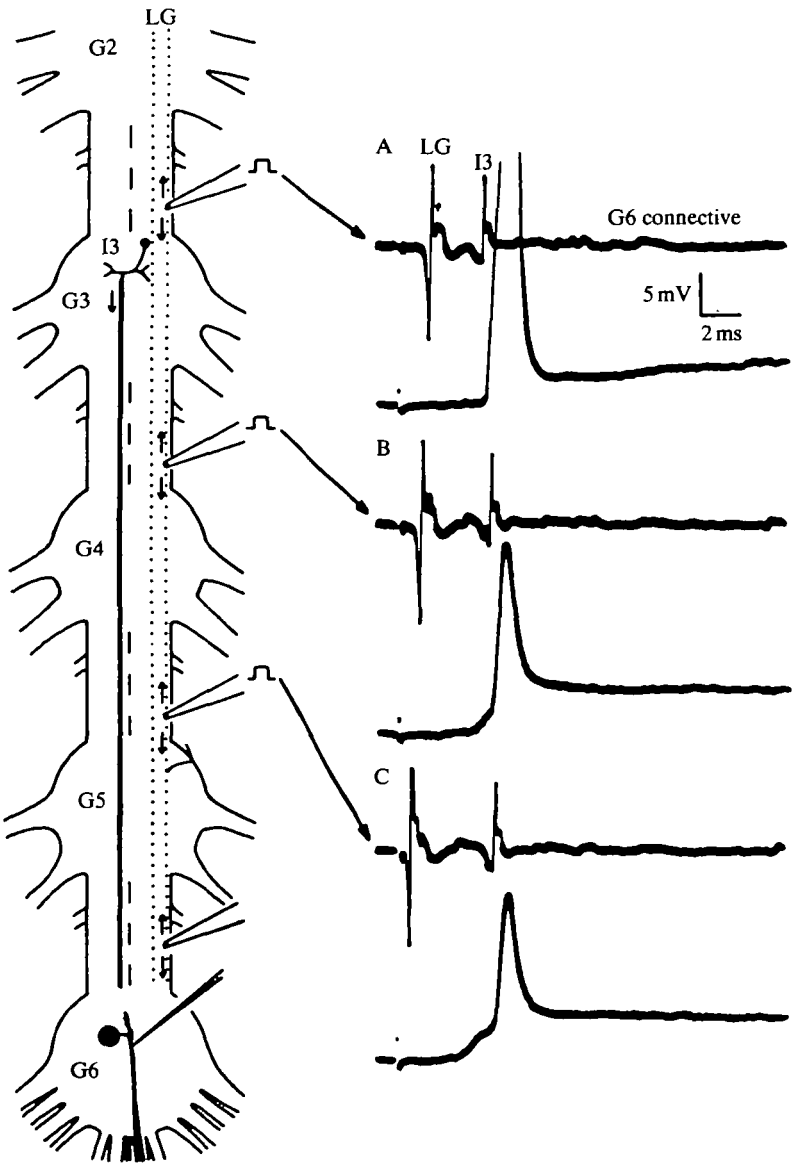

Fig. 6. The effect of the lateral giant (LG) on the telson motor giants (MoGs) depends on where the LG is activated. Inset: the experimental arrangement. We recorded from MoG6 in the neuropile and monitored the timing of the incoming impulses of LGs and I3s by recording extracellularly from the connective as it entered G6. We then stimulated the LGs just anterior to G3 (A), G4 (B) and G5 (C). The top trace . the connective recording and the bottom trace is the intracellular MoG6 recording.

Table 1. *Results of anterior activation of lateral giants (LGs) on the motor giants (MoGs) in G6*

Preparation	MoG not activated	MoG activated
Direct stimulation of LGs		
Isolated cord	3	9 (5)
Semi-intact	4	3 (2)
Minimal dissection	2	4 (2)
Sensory activation of LGs		
Semi-intact	0	2 (2)
Total	9	16

Figures in brackets are the numbers of experiments in which posterior LG activation was also tested. None of these fired the MoGs.

Behavioural analysis of LG tailflips

To investigate whether the site of impulse initiation in the LGs influenced the escape trajectory of intact animals, we studied giant-evoked tailflips using stroboscopic photography ($N = 4$) and EMG recordings of the PTF muscle ($N = 3$) in six animals (Fig. 7). We stimulated two animals by tapping one of three sites: the cephalothorax (to fire the MGs), segments 1 and 2 of the abdomen (to fire the LGs from a rostral site) and the tailfan (to fire the LGs from a caudal site). In all, 34 short-latency escape responses were obtained – roughly equal numbers from each of the three sites. In one animal, the LG axons were stimulated directly *via* electrodes implanted in segments 2 and 5. 19 responses were filmed; 9 in response to anterior stimulation and 10 to posterior stimulation. Finally, we electrically stimulated receptors *via* electrodes implanted in the lateral exoskeleton of the cephalothorax or second abdominal segment (100 trials, three animals).

The site of stimulation within the abdomen produced no difference in the form of the resulting escape response within any of the stimulus paradigms we used. Our failure to see differences was not due to the insensitivity of our measurements because we did see clear differences between tailflips elicited by stimulation of the cephalothorax (which recruits the MG interneurone) and the abdomen (which recruits the LGs), and we also saw differences between directly and reflexly evoked impulses (cf. Krasne & Wine, 1984; Dumont & Wine, 1986*b*).

These results indicate that, in intact animals, the telson flexor muscles do not contract in response to LG impulses, no matter where or how they are elicited.

The contradiction between the physiological and behavioural evidence cannot be resolved on the basis of the available evidence. The physiological results clearly indicate that the LG motor circuitry has the propensity to respond differently according to the site of impulse initiation. Our failure to demonstrate a behavioural expression of this propensity must mean that some condition in the intact animal either reduces the probability of telson MoG firing in response to rostrally initiated

LG impulses, or blocks their effect. Of course we cannot claim to have tested the animal under all conditions, so the possibility of site-specific behavioural outputs remains. This issue will be considered again in the discussion of the following paper.

However, if we assume that the stereotyped behaviour of the intact animal is the normal condition, then the physiological results must be artifactual. Even subtle artifacts could have important effects, because there is a delicate balance between the efficacy of excitation and inhibition of the telson MoGs. The timing which affects this synaptic efficacy depends upon the conduction velocities of the interneurones and the rise times of the synaptic potentials; efficacy also depends on the relative strengths of the synaptic potentials. All of these factors are independently capable of distortion.

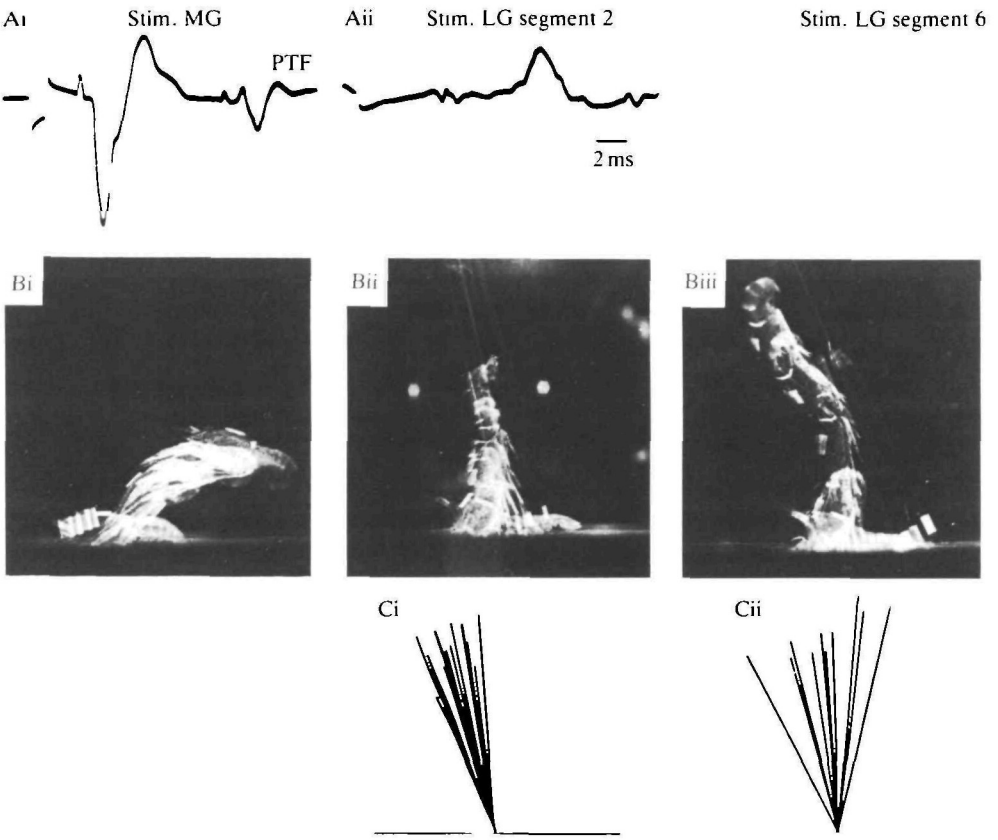

Fig. 7. Behavioural studies of giant-evoked tailflips. (A) EMG recordings from the posterior telson flexor (PTF) muscle during tailflips. When the medial giant (MG) tailflip was evoked by stimulating the cephalothorax, the motor giants (MoGs) fired, producing a twitch in the PTF. Stimuli to the second segment of the abdomen did not produce a response. (Bi) A tap on the cephalothorax produced an MG tailflip that propelled the animal backward. Taps to the anterior (Bii) and posterior (Biii) abdomen produced the same pattern of movement, a typical LG-mediated response, which flipped the animal forward. (C) Summary of 23 tailflips in one animal produced by stimulating the second or fifth abdominal segment. Stimuli at both locations resulted in the same movement.

DISCUSSION

The telson MoGs differ qualitatively from their anterior homologues in that they receive excitatory input from I2 and I3, the I3 input alone being sufficient to fire them. However, the inputs from these cells are rendered ineffective by central inhibition that is also evoked by the LGs and MGs (Kramer *et al.* 1981*a*,*b*; Fig. 4).

Prior to identification of these motor neurones as telson MoGs, the following explanation for this curious arrangement was put forward. The I2 and I3 contacts with the telson flexor motor neurones are used during non-giant flexion, thus these connections are functionally significant. The LGs and MGs fire I2 and I3 for reasons other than the excitation of telson flexors, such as control of uropod motor neurones. However, since flexion of the telson would distort the LG tailflip pattern, the I2s and I3s must be functionally disconnected from the motor neurones during LG tailflips. Hence the inhibition (Kramer *et al.* 1981*b*; Krasne & Wine, 1984). This provided a purely functional explanation for the observed connections.

This functional explanation is consistent with the facts as they were known then, but the identification of the telson MoGs and FFs, and our new knowledge about intersegmental differences in flexor premotor connections, supply a new context which causes us to question a purely functional interpretation of the MoG connections. We can think of four problems with the explanation as it stands.

The first problem is that the above explanation requires that the I3-to-telson MoG connection be used during non-giant tailflips. We now know this to be unlikely, since the anterior MoGs, which share many sources of inhibition with the telson MoGs, are inhibited during non-giant tailflips (Kramer & Krasne, 1984), implying that the telson MoGs would be too.

A second problem is that the telson FFs are not inhibited by giant commands, even though they are excited by I2 and I3 (Dumont & Wine, 1986*b*). Why should inhibition spare the telson FFs, whose firing during an LG tailflip would, like firing the telson MoGs, be detrimental?

A third problem with the purely functional explanation is that the anterior MoGs and the telson MoGs both receive command-derived inhibition, and although there are slight differences in the timing and form of the IPSPs, at least one pathway, from the interganglionic MoGI (Wine, 1977), is shared (Dumont & Wine, 1986*a*). However, the anterior MoGs receive no input from I2 or I3.

A final and particularly difficult problem for a purely functional explanation is that excitation of the telson MoGs by the I3s would be compatible with an MG tailflip, yet the MGs also inhibit the telson MoGs *via* the same inhibitory pathway that the LGs use.

The weight of the evidence is against the I3-to-telson MoG synapse being active in non-giant tailflips. We also know that it cannot be effective in the MG tailflip, as the I3 input is not only superimposed on a large IPSP, but arrives when the cell is still refractory after having been fired by the MGs. We also went to some length to examine the possibility that the I3 input might be suprathreshold during LG-activated tailflips. Again, the I3 input is superimposed on an IPSP, but since the end

result depends on a delicate balance of excitatory and inhibitory input, the possibility of activation remained. However, the evidence from intact animals suggests that this does not occur naturally. Thus, we have been unable to establish a functional reason for the I3-to-MoG connection in this segment. Can we suggest an alternative reason for the differences that have arisen between the inputs to the MoGs of the telson and the anterior segments?

While there appears to be no functional difference between the telson MoGs and those in G4 and G5, there is a conspicuous morphological difference which may provide a suitable starting point for an alternative explanation. In anterior segments the dendrites of the MoGs are confined to the connectives caudal to the ganglion, at the point where the third nerve arises. However, in G6, in the absence of caudal connectives, the dendritic branching of the MoGs lies within the neuropile. The CDIs I2 and I3 have branches only within the neuropile area of the ganglia they pass through. Therefore, the sixth ganglion is the only one in which I2 and I3 have the opportunity to synapse with MoGs. Rather than arguing a reason for the presence of the I3-to-MoG connection in the sixth ganglion, we have an explanation for its absence in G4 and G5. The presence of the I3-to-MoG synapses in G6 may now be understood as a remnant of a time when the tailflips were less specialized and connections between premotor and motor neurones less restricted. Also, it has been suggested that I2 and I3 may be homologues of MG (Miller, Hagiwara & Wine, 1985), and so they may retain the ability to form synapses with the MoG when the opportunity arises. The properties of the I3-to-MoG and MG-to-MoG synapses are certainly very similar.

A similar hypothesis has been put forward to explain the differences in connections made by identified neurones in different individuals of *Caenorhabditis elegans* with the same genotype (White, Southgate, Thomson & Brenner, 1983). There it was found that one feature governing synapse formation was simply which cells were available for contact. Similarly, studies of development in insects (Bastiani, Pearson & Goodman, 1984; Murphey, Bacon, Sakaguchi & Johnson, 1983) make the point that the first stage in development of neuronal connections is the elaboration of cell processes in the correct neighbourhood. Interestingly enough, the MoGs' synaptic regions in anterior segments already lie outside of the developing neuropile at about 60 % of development. One unsatisfying aspect of this explanation is that it is not clear why the inhibitory inputs to anterior MoGs should have been retained during the migration of the synaptic sites from the ganglion to the connective, while the I3 and I2 inputs were lost.

It is not yet possible to substantiate such speculation. However, the stereotyped nature of crayfish escape behaviour, the simplicity of the circuitry controlling it, and the range of similar circuits found in different species allow investigations that can at least show that such a possibility is plausible. Thus, if the movement of the MoG dendrites into the connective in anterior segments was an important factor in isolating these motor neurones from premotor interneurones, then such contacts might be found in other situations in which the MoGs do have ganglionic dendritic

arborizations, such as the thoracic ganglia of the crayfish (Crabtree & Sherman, 1980).

The overall conclusion of this paper is that adaptive changes in nervous systems are virtually certain to entail a large set of inextricably interconnected alterations, only some of which are useful. The constraints imposed by linkages in developmental mechanisms are probably especially important in this regard. Ineffective connections have been noticed with increasing frequency as techniques have improved. For example, the motor neurones innervating the muscles of the sound-producing spiracle in *Gromphadorhina portentosa* fire at low frequency in synchrony with the normal respiratory movement of the unspecialized spiracles, but the effect on the muscle is insufficient to produce movement. The weak output is presumably a vestige of the cell's previous involvement in respiration (Nelson, 1979). A similar conclusion has been reached by a different approach. In the study of the development of *Caenorhabditis elegans*, Sulston, Schierenberg, White & Thomson (1983) found many inefficient developmental processes which they called 'developmental fossils'.

Finally, it should be pointed out that our suggestion that certain features have no functional significance is in reference to their immediate expression in behaviour. The weak or inhibited connections we encountered provide a diffuse pattern of weak connections between many parts of the nervous system. Selective strengthening of such connections could be important as a substrate for further evolution, and might also underlie many forms of learning.

We wish to thank Dr M. Kirk for his help on the study of the local inhibitors of the MoG, and G. Hagiwara for her assistance with all phases of the work. Also, we are grateful to Dr F. Krasne for his comments on the manuscript, and J. Ruby for her help in preparing it. This study was supported by NSF grants BNS 80-15583 and BNS 81-12431 to JJW and a Stanford University Biological Sciences Department Teaching Assistantship to JPCD.

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