THE TELSON FLEXOR NEUROMUSCULAR SYSTEM OF
THE CRAYFISH

III. THE ROLE OF FEEDFORWARD INHIBITION IN SHAPING A
STEREOTYPED BEHAVIOUR PATTERN

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SUMMARY

1. The telson flexor system is homologous to the fast flexor system of anterior
ganglia (Dumont & Wine, 1986a), but important differences exist in connections to
the telson motor giants (MoGs) (Dumont & Wine, 1986b). In this paper, we
describe additional differences in connections to the telson non-giant fast flexor (FF)
motor neurones and to the telson flexor inhibitor (FI).

2. The telson FF motor neurones in ganglion 6 (G6) receive inputs similar to
those in G4 and G5 (Miller, Hagiwara & Wine, 1985). The escape command
neurones (lateral giants, LGs, and medial giants, MGs) in common provide weak
disynaptic input via the telson segmental giant (SG6), and relatively strong tri-
synaptic input via SG2, SG3 and the corollary discharge interneurones I2 and I3.
There may also be some direct input from the MGs, but it, as well as the connections
from SG6, appears to vary in different preparations.

3. The compound PSP produced in telson FFs by a single LG or MG impulse
was suprathreshold in only five of 55 experiments in isolated abdominal nerve cords,
but the probability that a motor neurone would fire increased with additional giant
axon impulses, showing that temporal summation of excitation outweighed the
possible recruitment of inhibition. Firing probability was higher in semi-intact
preparations, where at least one posterior telson FF was fired by a single LG impulse
50% of the time. As was pointed out previously (Dumont & Wine, 1986b), telson
flexion would disrupt the behaviour pattern expected from LG commands.

4. Two pathways of feedforward inhibition were found which prevent such
disruption. The sensory input that recruits the LG also recruits powerful feed-
forward inhibition of the telson FF motor neurones, which reduces the probability
that they will be fired by the LG. The same sensory stimulus also evokes inhibition
of FFs in G5, excitation of FFs in G2 and G3, and mixed excitation and inhibition of
FFs in G4. In addition, the telson FIs fire at short latency during LG-mediated
tailflips. This occurs because the telson FIs are excited by sensory input. In fact, the
firing threshold of the telson FIs to sensory input is lower than that of the LGs, at
least for electrical stimulation of nerves. When the LGs do fire, they produce

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additional excitation of the FIs. The telson FIs also are excited by the LGs but not by the MGs. In contrast, the anterior homologues of the telson FIs receive equivalent, delayed excitation from both MGs and LGs, and weaker sensory input, so that they tend to fire only after the peak of flexion (Wine & Mistick, 1977).

5. The predicted net effect of these connections is that the telson flexor muscles should not contract during naturally elicited LG tailflips, and this is consistent with observed behaviour. The results can be interpreted as providing additional examples of potentially maladaptive central connections which are not expressed in behaviour because of feedforward inhibition.

INTRODUCTION

This paper continues our intersegmental comparison of the telson flexor system by documenting differences in pathways to the non-giant flexor motor neurones and to the flexor inhibitor. In this study we encounter two new forms of the odd phenomenon in which a single source evokes concomitant excitation and inhibition of a motor pathway, so that the excitation is prevented from being expressed in behaviour. The previous study (Dumont & Wine, 1986b), described how indirect, central excitatory input from the giant command cells to the telson motor giant was cancelled by a central inhibitory pathway. Inhibition was recruited by both the LG and MG escape command cells; different motor output for the two command cells occurs because direct connections exist from the MGs to the motor giants. In this study, we again encounter an anomaly: the LGs excite motor neurones (the telson FFs) whose output would interfere with the behavioural pattern observed when the LGs fire. Here again the behaviour pattern is prevented by inhibition, but in this example the inhibition is not derived from a central pathway, but is instead recruited by sensory pathways.

MATERIALS AND METHODS

Three types of preparation were used. The closest of these to the animal’s natural condition was the minimally dissected preparation. Crayfish 4–6 cm long were restrained, ventral side up, in Van Harreveld’s (1936) solution. The ventral cuticle over the nerve cord was removed from the abdomen. The fast flexor nerves of G1–G5 were cut to prevent flexion of the main axial muscles. The first and second nerves of (usually) G3 and G4 were tied with thread and then cut. These two ganglia were then held dorsal side up by securing the threads. This allowed us selectively to stimulate or record from the LGs or MGs with suction electrodes. The cuticle on which the posterior end of the ventral telson flexor (VTF) muscles insert was freed from the surrounding tissue, and the VTFs were pinned back to allow access to the sixth nerve (N6) and the anterior telson (AT) and posterior telson flexor (PTF) muscles. The nerves of the sixth ganglion were left intact for recording motor neurone activity from N6 G6 or telson flexor muscle activity. Sensory nerves (other than those mentioned above) were left intact, as was the thoracic–abdominal connective and the circulation.
The other two preparations (semi-intact and isolated cord) have been described in detail elsewhere (Dumont & Wine, 1986a). Briefly, the semi-intact preparation was similar to the above except that G6 was stabilized by pinning it to a wax-covered platform and the sheath was treated with pronase for 15 s for recording from somata. The thoracic–abdominal connective was cut if we intended to evoke LG activity with sensory input, since in restrained animals there is strong descending input of sensory activation of the LGs (Krasne & Wine, 1975).

The isolated nerve cord preparation consisted of the abdominal nerve cord attached only to one PTF muscle by N6. The ventral sheath over G6 was removed for neuropilar penetration.

RESULTS

Inputs to the telson FF motor neurones

Complex synaptic inputs are generated in the telson FF motor neurones by activity originating from three sources: the giant escape command axons, sensory pathways and the non-giant escape premotor system. We will deal only with giant- and sensory-evoked input in this paper, although some interneurones are common to all three systems.

Pathways for giant-evoked input to the telson FFs

In the five anterior abdominal ganglia, three pathways exist from the giant escape command cells to the non-giant fast flexor motor neurones (FFs). The relative importance of these pathways changes in a segment-specific fashion (Fig. 1A). The monosynaptic pathway (not shown in Fig. 1A) is weak and variable in all segments (Roberts et al. 1982; Miller et al. 1985). The disynaptic pathway *via* the segmental giants (SGs) is the main pathway in G1–G3; in those ganglia the SGs fire the FFs *via* one-to-one electrical synapses (Roberts et al. 1982). In G4 and G5, the SG input to the FFs is usually subthreshold (Miller et al. 1985). Trisynaptic pathways, *via* SG2–I2 and SG3–I3, provide additional input to FFs in G4 and G5 (Kramer, Krasne & Bellman, 1981a; Kramer, Krasne & Wine, 1981b; Miller et al. 1985). We found that the telson FFs receive input from all these pathways, but the inputs differ quantitatively: the SG input is much weaker and the I2/I3 input is slightly stronger than in anterior ganglia, so that the overall efficacy of the input is reduced. When measured in the neuropile, the mean peak amplitude of the compound giant-evoked EPSP was 17·5 mV (s.d. = 4·5 mV, N = 17).

The inputs from the various giant-evoked premotor neurones were distinguished by firing corollary discharge interneurones (CDIs) individually, by repeated high-frequency firing of the giant command cells to induce CDIs to drop out, or by collision experiments (Fig. 2). When this was done we were able to categorize the inputs as follows (all amplitudes measured in neuropile).

1. Mono- and disynaptic inputs. The short-latency input to the FFs had a mean amplitude of only 5·8 mV (s.d. = 5·5 mV, N = 12). This short-latency component contained the ipsilateral SG input of 2·4 mV (s.d. = 2·6 mV, N = 8).
remainder of the short-latency input (approx. 3 mV) was due to a route or routes other than the SG; one may be monosynaptic (Fig. 2).

(2) Trisynaptic input. The remaining input comes from the I2s and I3s (interneurones with cell bodies in G2 and G3, respectively), which synapse on all telson FF motor neurones. When recorded in the neuropile, the mean amplitude of the summed EPSPs from the I3s was 13.3 mV (s.d. = 4.5, N = 17), five times the amplitude of the SG input, and the amplitude of the I2 EPSPs was 6.1 mV (s.d. = 2.3, N = 10). Therefore, for telson FFs, the major portion of the giant-evoked input is via I2/I3 instead of the SGs. A quantitative comparison of the absolute amplitudes of I2/I3 inputs to telson FFs reported here and those found for

Fig. 1. Gradient of excitation in fast flexor (FF) motor neurones. (A) Diagram of connections between lateral giant (LG) and medial giant (MG) and the FFs in the different abdominal ganglia. In anterior ganglia (G1–G3), the FFs are fired via the strong synapse from the segmental giant (SG). In more posterior ganglia this becomes weaker, and the FFs receive more of their input from I2 and I3. (After Miller, Hagiwara & Wine, 1985.) (B) Neuropile recordings of LG input in FFs from G2, G4 and G6. Dotted line indicates the time the LG spike reached the ganglion, based on extracellular recordings. (C) Graph of probability of activation of FFs by LGs or MGs for G2–G6. (Data for G2–G5 from Miller et al. 1985.)
Fig. 2. Selected evidence for mono-, di- and trisynaptic connections between the giant axons and the telson fast flexors (FFs). (A) Diagram shows the set of inferred connections. (B) In most experiments, the shortest latency input from the giants is disynaptic, via the segmental giant (SG). Here, superimposed traces from cell body recordings show the effect of stimulating the contralateral medial giant (MG) and then the ipsilateral segmental giant (SG). Note that the initial response to MG stimulation is entirely due to its recruitment of SG, since direct stimulation of SG gives an identical early response. (C) Major giant-evoked input to telson FFs is usually trisynaptic, via I2/I3. (i) A typical response recorded in the neuropile: the early component is via the SG, the second component is via I3, and the third component via I2. (ii) Unusual example of direct input from MG axon. Here firing the ipsilateral MG produced a larger and faster-rising early EPSP that may represent a direct connection.

anterior FFs in a previous study (Miller et al. 1985) is not possible, because the latter were based on soma recordings. However, it is clear that the relative strength of the I2/I3 input is much greater in G6 than in G4 and G5. This is demonstrated by the three neuropile recordings shown in Fig. 1B. The FF in G2 receives only a suprathreshold short-latency input, in G4 the input is subthreshold and the later components from I2 and I3 are apparent, while in G6 the short-latency component has almost disappeared while the delayed I2 and I3 inputs are much more marked.

The early inputs to the FFs show considerable variation in amplitude, as indicated by the large standard deviations. This is probably partly due to variations in the position and quality of the recordings, but that cannot be the whole explanation since the short-latency input was more variable than the other components of the EPSP (Fig. 2). We saw no reliable differences in the relative amplitudes of the components among different telson FFs, and therefore we have pooled the results. However,
because of our small sample, we may have overlooked real differences in input to different FFs.

To summarize: telson FFs receive a compound EPSP when the giant axons are directly fired. The input differs from that seen in FFs of anterior ganglia in that the early components are weaker and the later components stronger.

**Efficacy of giant axon input to the telson FFs**

The giant interneurones excite the FFs monosynaptically, disynaptically and trisynaptically. What is the net effect of all of this input on firing probability? In G1–G3, single impulses directly evoked in the LG or MG fire the FFs with about 90% reliability, while in G4 and G5 the probability of firing is reduced to less than 40% (Miller et al. 1985). We have established that this trend continues into G6 (Fig. 1B,C). When telson FFs were recorded intracellularly, only 9% (N = 55) produced action potentials in response to a single impulse in the LGs or MGs. If two LG impulses were used (two or more impulses were commonly observed in intact crayfish, Wine & Krasne, 1972), the frequency of response increased to 18%.

These results were extended by extracellular recordings from a variety of preparations, including minimally dissected preparations (see Materials and Methods). Such recordings were begun within 1 h of the start of the dissection (compared with about 3 h for intracellular recordings), and did not involve cell penetration. Recordings were made from the PTF branch of nerve 6, which contains four FFs. Under these conditions, we detected firing in 50% of the preparations tested (N = 20). The probability of any given telson FF being fired was 21% (assuming the probability is the same for all telson FFs). The probability of response was the same for LG or MG. Since the recordings were extracellular, these figures assume that the FIs were not being recruited. This seems a safe assumption, especially for MG stimulation. Even if the FIs were sometimes being fired and mistaken for FFs it would not alter the main conclusion, but would simply mean that the probability of firing a telson FF was lower than we estimated.

To summarize: in our experiments, the telson FFs were fired erratically by directly evoked LG impulses.

**Sensory input to the telson FFs and to FFs in other ganglia**

Direct activation of the LGs can fire some of the caudal FFs, yet the potentially disruptive effects of this on the movement produced are not seen in the naturally evoked behaviour. One possible reason for this difference might be an effect of the sensory stimulus itself, since this is present in the naturally evoked behaviour but not with direct stimulation of the LG. We therefore examined the effects of sensory input on the abdominal FFs. The results were as follows. In G2 and G3, the FFs were excited via polysynaptic sensory pathways when the abdominal sensory nerves were electrically stimulated (Fig. 3A). In contrast, FF motor neurones in G5 and G6 were inhibited when the same sensory nerves were stimulated (Fig. 3B). The effect of sensory nerve stimulation on the FFs in G4 was ambiguous, and may be a mixture of excitatory and inhibitory input.
These effects of sensory input were revealed in the set of experiments which is summarized in Table 1. FFs from the different ganglia were impaled in the neuropile and the giant interneurones were stimulated in the connective, sometimes with a group of other interganglionic premotor neurones that also lie in the dorsal cord (Kramer et al. 1981b). In the case of the anterior FFs, which received supra-threshold input from the giant axons, the cell was hyperpolarized to keep it from firing. Once a sizeable EPSP had been obtained, we recorded the effect of superimposing it on a sensory-evoked PSP. If the sensory input was excitatory, the PSPs would summate. If it was inhibitory, the large, giant-evoked PSP would be shunted. We used this approach because the IPSPs are depolarizing, which made it difficult to discriminate them from EPSPs with confidence, although they do have different time courses.

The latencies of the sensory-evoked PSPs in the FFs varied, and were generally shorter with more intense stimuli. Latencies as short as 5 ms were frequently recorded. These are fast enough to interact with the command-derived input during escape behaviour. These effects of sensory input could provide a means of correcting the apparently disruptive effects of LG–FF connections in caudal ganglia.

![Figure 3](image_url)

Table 1. Effect of sensory root shock on fast flexor (FF) motor neurones

<table>
<thead>
<tr>
<th>Stimulus site</th>
<th>N</th>
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<th>G2</th>
<th>G3</th>
<th>G4</th>
<th>G5</th>
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<tr>
<td>FF2</td>
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<tr>
<td>FF4</td>
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<td>FF6</td>
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+ = excitatory, − = inhibitory.
Two problems with using electrical shocks to nerves instead of natural sensory stimuli are that shocks recruit different modalities of afferents in unnatural and largely unknown combinations, and they also antidromically fire motor axons in mixed nerves. We do not think these problems are major, since we saw similar results from stimulating various nerves, which makes it unlikely that any specific motor neurones or proprioceptive pathways are exclusively involved. Mechanosensory afferents, in contrast, are present in large numbers in all abdominal sensory nerves except the posterior intestinal nerve.

To summarize: abdominal sensory input reinforces the partial pattern of flexor output associated with LG firing.

**Inputs to the telson flexor inhibitors**

**Giant-evoked input to the telson FIs**

In anterior abdominal ganglia, the FIs receive equivalent inputs from the MGs and the LGs (Fig. 4Ai). The input is polysynaptic, multicomponent and occasionally suprathreshold (Wine & Mistick, 1977), initiating one or a series of spikes after a delay (7.5–25 ms) which is shorter in G4 and G5 (Uyama & Matsuyama, 1980). The input to the G6 FIs differed from this in two ways. First, the later, polysynaptic phase is weaker in telson FIs, and in many cases it appeared to be absent (compare Fig. 4Ai and 4Aii, both of which are soma recordings). The early phase (amplitude = 2.7 mV, \( N = 9 \), in G6 soma recordings) appears comparable. Second, comparison of Fig. 4Aiii (neuropile recording) with Fig. 4Ai shows that in the telson FI the LG and MG inputs are no longer equal, the MG input lacking the large, early LG-evoked EPSP. The input to the telson FIs from a single LG impulse was subthreshold in our experiments, but trains of LG impulses caused the FIs to fire, whereas trains of MG impulses did not. The imbalance of command inputs makes it more likely that the FIs will be recruited during LG, rather than MG, tailflips. These connections are summarized in the circuit diagram in Fig. 4C.

**Sensory input**

FIs in anterior ganglia receive rather weak sensory input (Wine & Mistick, 1977). In contrast, the telson FIs receive much stronger sensory input; large EPSPs are seen in response to stimulation of sensory nerves anywhere within the abdomen. Sensory input is particularly strong for FI7, which is activated by stimuli less intense than those required to activate the LGs (Fig. 5). While FI6 appears to be less sensitive to sensory stimuli, at least in our preparations, the combination of sensory and subthreshold LG input is sufficient to fire it (Fig. 6).

To summarize: the net effect of central and sensory feedforward connections to the telson FIs should evoke their early firing during naturally elicited LG tailflips, which is an additional mechanism for ensuring the spatial pattern of flexor output associated with LG firing.
Fig. 4. Input to telson flexor inhibitors (FIs). (A) Comparison of medial giant (MG) and lateral giant (LG) input to FIs in G3 and G6. The compound EPSP due to LG input is shown as a solid line, and the compound EPSP to MG input is shown as a dotted line. (i) In the soma of an FI in G3, the responses to MG and LG stimulation are, on average, identical; the variations shown here are no greater than trial-to-trial variations in response to repeated stimulation of one of the giant command axons. The input has two components: an early, direct input and a delayed input via the CDIs. (ii) Soma recording of LG input in G6. The late, corollary discharge interneurone (CD1)-dependent component is greatly reduced compared with more rostral ganglia (i). (iii) In contrast to the response in G3, the response in G6 to LG stimulation is much larger than the response to MG stimulation (neuropile recording). (B) Sensory input can fire FI7. (i) Responses to a series of shocks to the fourth nerve, which is an almost pure sensory nerve that innervates the anterior part of the dorsal telson. Four superimposed responses are shown to shocks of increasing intensity – at the highest intensity the cell spiked (asterisk). (ii) An antidromic impulse, shown for comparison. Responses in B were recorded in the soma. (C) Schematic diagram of altered central and sensory connections to telson FIs. These are functional and not monosynaptic connections.
Modification of LG tailflips by sensory input in semi-intact preparations

To examine the possible role of sensory inputs during LG tailflips, we recorded telson flexor motor neurones extracellularly and telson muscle fibres intracellularly in a semi-intact preparation. The semi-intact preparation should provide a closer approximation to natural behaviour than the more extensively dissected preparations. We first established baseline responsiveness of the FFs by directly stimulating the LGs in the connective and recording motor output in the sixth nerve and the PTF muscle (Fig. 7). We evoked paired LG impulses to produce a reliable FF response, since other experiments showed that there is only a 10–20% chance of single LG shocks evoking activity in telson FFs. Paired impulses approximate the natural condition mentioned earlier, since two LG impulses are a common response to tactile stimuli (Wine & Krasne, 1972). In the experiment shown, one FF was fired by the paired LG impulses (Fig. 7A). It reliably produced a facilitating EPSP that was suprathreshold in most of the muscle fibres of the rostral dorsal part of the PTF muscle. To test the effect of sensory input, the second nerve of G4, which contains afferents that excite the LG, was stimulated at the same time as the LG. This caused the pattern of motor activity in the sixth nerve to be radically altered: the telson FF motor neurone did not fire whereas the telson F1s did (Fig. 7B). Both sensory effects depressed with repetition. When the sensory and LG stimuli were repeated 1 s later (Fig. 7C), the FF was again recruited, but so was an FI. The IPSP produced by the FI shunted the EJP from the FF and blocked the muscle spike. In subsequent trials, the sensory input became ineffective and the pattern of outputs reverted to that of the LG alone.

While we do not yet have proof that mechanosensory afferents are responsible for this effect, they are very likely to be involved. Similar effects were obtained by stimulating any afferent-containing nerve in the abdomen. To our knowledge, only mechanosensory effects are so widely distributed. In particular, the effect was seen when the purely sensory fourth or fifth nerves of G6 were stimulated, so it clearly does not depend on ortho- or antidromic stimulation of motor neurones in the mixed nerves. In soma recordings from telson FFs in preparations having intact sensory nerves, PSPs were seen in response to blowing on the surface of the saline.

DISCUSSION

General considerations

The central connections that sensory and command pathways make with telson flexor efferents differ quantitatively and qualitatively from connections made among homologous neurones in anterior ganglia. In addition, the number and form of the segmental premotor neurones is changed. In attempting to understand the origin and significance of the differences, we begin by assuming that the present abdominal ganglia evolved from a chain of prototypical ganglia that were either identical to one another or much more similar to each other than to present-day ganglia. A second assumption is that the ancestral ganglia were highly interconnected and complex, perhaps having even more neurones than present-day ganglia. Those assumptions
Fig. 5. Sensory input evokes an action potential in flexor inhibitor (FI7) at intensities lower than those required to activate the lateral giant (LG). (A) A high-intensity shock to a sensory nerve (N5 G6) fired the LG (connective recording, bottom trace) and FI7 (top trace, N6 G6). FI7 was identified by the depolarizing IPSP it produced in the dorsal posterior telson flexor (PTF) muscle (middle trace). (B) A lower intensity stimulus did not activate the LG, but was suprathreshold for the FI. (C) The sensory input depresses with repetition, so that the same intensity nerve shock as in B failed to produce a response when applied 1 s later.

Fig. 6. Sensory activation of lateral giant (LG) activates (flexor inhibitor) FI6 (neuropile recordings). (A) Activation of the LG in the connective produced a subthreshold EPSP in FI6. Note that this EPSP appears to consist exclusively of short-latency input. (B,C) Activation of LG with sensory nerve (N4 G6) shock. (B) First stimulus, LG and FI6 are activated. (C) Fourth stimulus at 1 Hz, the LG response fails as does the FI6 response.

Fig. 7. Sensory input can modify the motor pattern generated by the lateral giant (LG). Top traces, recordings of LG impulses in the connective; middle traces, recordings of posterior telson flexor (PTF) motor neurone spikes in N6 G6; bottom traces, intracellular recordings from PTF muscle fibre. (A) Stimulation of LGs alone fired a fast flexor (FF) motor neurone, which in turn fired the muscle fibre. (B) Stimulation of LGs plus a sensory nerve blocked the FF spike, and caused a burst of three spikes in the flexor inhibitor (FI). (FI spikes were identified by their effect on the muscle fibre.) (C) The same stimuli 1 s later. The sensory effect is now weaker. The FF fired, but the EJP in the PTF muscle fibre was shunted by the IJP from FI.
are based on comparative and developmental evidence (Keyser & Lent, 1977; Lawrence & Morata, 1983; Loer, Steeves & Goodman, 1983; Schram, 1982).

If a change occurs in one part of a complex and highly interconnected system, it will cause widespread changes in other parts. When considered individually, each change may be beneficial, neutral or harmful; but if the net effect of the changes is improved performance of the system, it pays to maintain the changes. Our third assumption is that evolution proceeds in just this way.

To what extent can changes in nervous systems be made specifically and independently? We know that there is not enough genetic information to specify each synaptic connection individually. Thus, one can imagine that when some unknown epigenetic rule dictates formation of a behaviourally advantageous connection between two neurones, it may also commit the cells to contacting other neurones which, in an optimally designed system allowing for independent connections, it would not contact. Functional validation of synapses, such as by the scheme proposed by Hebb, is one strategy for enormously enhancing the resolution of connection specificity in a nervous system. This strategy is found throughout the animal kingdom, including the abdominal nervous system of crayfish (F. B. Krasne, in preparation). However, functional validation is not a good strategy for building an escape circuit which must operate optimally the first time it is challenged. For that reason the crayfish escape circuit is presumably shaped more by evolution than by learning. Under these circumstances a considerable number of ‘junk’ connections might be expected.

Given that expectation, how is it possible to decide if a particular connection is functionally significant? The functional significance of a particular connection is difficult to determine. In principle, one would like to see the connection operating in normal behaviour and then observe a detrimental change in the behaviour when the connection is selectively eliminated. In practice such experiments are rarely possible, functional significance is commonly assumed, and the burden of proof has been shifted onto those who would deny such significance. However, whereas it is often difficult to establish functional significance, it is virtually impossible to prove that a synaptic connection is nonfunctional, for no matter what one does, it is always possible to imagine an environmental circumstance, different from any that were tested, which might be the circumstance in which the connection makes its significant contribution.

With these general considerations in mind, we review the differences in premotor organization among the serially homologous abdominal ganglia, and attempt to offer a plausible rationale for some aspects of the present plan.

The SG-to-FF connections

From the second to sixth ganglia, the probability of an SG impulse firing any given FF motor neurone declines continuously but not linearly: the largest drop, from about 60% to about 20%, occurs between G3 and G4. If there is a way to argue that this gradient is an optimal design, it has escaped us. A strictly adaptationist explanation would be that the production of the correct motor output requires such a
Segmentally homologous neurones in the crayfish III 307

graded decline in synapse strength. However, this is not the case. The SGs are fired by both the MGs and LGs, but neither command neurone produces any segmentally graded motor output. The MGs evoke flexion of all segments, whereas segments 4–6 remain extended during LG tailflips. Therefore, the progressively weaker connections are an embarrassment for both command cells. Even if these connections represent a compromise between the requirements of MG and LG tailflips, one would expect a stepwise change in connection strength between ganglia 3 and 4, not a graded change.

An alternative explanation may be that the graded change represents the best approximation to a stepwise change that can be achieved. If the developmental processes which led to the formation of the SG-to-FF connection are the same in all ganglia, then altering the strength of the connections in some ganglia but not others may not be easy. This developmental restriction might hamper the evolution of a discontinuous change. While genetic specification of segments appears to be quantal in nature (Lawrence & Morata, 1983), the effects on the phenotype, at least in the abdomen of arthropods, need not be. Thus in the grasshopper, the M cell homologues show graded and probabilistic changes in form and function, spread over several ganglia (Bate, Goodman & Spitzer, 1981).

The persistence of these potentially maladaptive connections can be understood if they are not expressed in the behaviour of the animal and therefore cannot be selected against (see below). This immunity from selective pressure may also explain the variability in the strength of the SG-to-FF synapse, and indeed the other short-latency components of the MG- or LG-evoked EPSP. Such an explanation has also been suggested for the variability in connections of a large visual interneurone (the DCMD) with thoracic motor neurones in the locust. The main effects of the DCMD are expressed indirectly via interneurones, while the direct connections of the DCMD are weak, often absent, and are made nonspecifically with two sets of antagonistic motor neurones. All of these features suggest that they are nonfunctional (Pearson & Goodman, 1979).

No matter which argument we favour, it does not help us reconstruct the direction of evolution in this system. There are three possible scenarios. One is that all the SG-to-FF synapses were once strong, another is that they were all once weak, and the third is that they were once intermediate, as in G4.

Interspecies comparisons could, in principle, help answer such questions, but no other crustacean has been investigated in detail. In the best example so far, Anaspides tasmaniae, a crustacean which diverged from the ancestors of the crayfish about 300 million years ago, was studied by Silvey & Wilson (1979). Anaspides has many features which are considered primitive, such as a relatively undifferentiated thorax and abdomen. It also has LGs that are strikingly similar to those of the crayfish and are presumably homologous. In these animals, activation of the LGs causes flexion of all segments, but it is not known whether the LG-to-FF connection is via SG homologues.

Whatever way it evolved, the SG-to-FF pathway is a clear example of a graded segmental difference, yet these same ganglia also show an all-or-none difference in
synaptic connectivity between the LGs and MoGs (Dumont & Wine, 1986b). The coexistence of both continuous and discontinuous segmental specifications may provide insight into the evolutionary and developmental processes that shape these circuits. There are, of course, many differences which might explain this more abrupt change in properties of the LG-to-MoG synapses. These synapses are faster, more specialized and, since they lie in the connective rather than in the neuropile, may be developmentally simpler.

**Sensory input to the flexor efferents**

We have shown that sensory nerve shocks which activate the LGs also cause feedforward effects on the FFs and FIs. For the anterior FFs the effect is excitatory and complements the strong LG-evoked excitation. For the posterior FFs, the effect is inhibitory and therefore counteracts the excitation caused by the LGs (Fig. 6B). This brings the pattern of outputs from the FFs during LG-evoked tailflips into line with the MoG output pattern, and is consistent with the observed behaviour. Sensory excitation of the FIs, at least in G6, complements this process: sensory feedforward fires the FIs prior to firing the LGs (Fig. 5). Thus, even if the FF motor neurones should escape sensory inhibition, their effects will be counteracted by peripheral inhibition.

These feedforward effects of sensory input fit in well with the results of behavioural experiments. Evoking LG tailflips in intact, freely moving animals produces different results depending on whether the LGs are activated with sensory input or by stimulating the cord directly with implanted electrodes (Krasne & Wine, 1984). When tailflips are evoked with sensory input, either by tapping the animal's abdomen or by passing current through electrodes implanted in the exoskeleton, the animal pitches up and forward. But when the tailflip is evoked by directly stimulating the nerve cord, the final body angle is slightly backwards (Fig. 8). The electrically induced tailflip is intermediate between the naturally evoked LG and MG tailflips, and is consistent with LG activation of some of the posterior flexor muscles. When the LGs are activated with sensory input, this does not happen, consistent with sensory inhibition of the more posterior flexor muscles.

We believe that these findings have implications for understanding how command neurones work and how this circuit evolved. The standard model of command neurone activity requires that activity in such a cell is sufficient to produce the complete behaviour pattern (Kupfermann & Weiss, 1978; Fig. 9). We have shown that firing the LG is not sufficient to produce the typical motor pattern. Instead, sensory interneurones, in addition to activating the command neurone, also feed forward to affect the generation of the motor pattern (Fig. 9).

Sensory modification of centrally generated motor patterns has been documented extensively, particularly in the locomotion of insects (Pearson, Fourtner & Wong, 1973; Reichert & Rowell, 1985). Indeed in the case of walking, which must be carried out over uneven surfaces, it must be essential. However, in such cases sensory input usually operates in a feedback role, whereas in this ballistic system it operates as feedforward. Sensory feedback compensates for errors in movement or
irregularities in the environment; that is, it makes a stereotyped central pattern generator more flexible. In the escape system, sensory input plays a very different role: it appears to make a potentially variable central programme more stereotyped.

We can see no functional argument for why the LG does not code the movement accurately on the basis of central connections. Between G3 and G4 the LGs become completely disconnected from the MoGs (with the exception of the indirect connection in G6 that is centrally inhibited). If the same switch occurred with the FFs the behaviour should be accurately and consistently produced. Yet no clear switch occurs, and instead sensory input is required to ensure the appropriate form of the LG tailflip.

We suggest the following scenario. At some point in the past, MG and LG tailflips were more similar than they are now, as seems to be the case in *Anaspides tasmaniae* (Silvey & Wilson, 1979). Selective pressure then favoured a reduction of flexion in posterior segments in response to abdominal stimuli. Since either weakening the command input or strengthening the inhibitory effect of sensory input would work,
both strategies were employed. Once the present highly efficient behaviour had evolved, there was no longer any means of selecting a different arrangement, which would only produce the same end result. This scenario does not exclude the possibility that at one point in the evolution of this system the LG-mediated tailflips were more variable than now, nor that the position of the stimulus could affect the final behaviour, either through sensory pathways or the position-related effects outlined earlier (Dumont & Wine, 1986b).

The major alternative explanation for the results would suggest a functional advantage for every connection found, and would argue that we have simply not employed the right conditions to demonstrate the full utility of the system. We concede the point, but find the evolutionary arguments more compelling. Similar conclusions have been reached many times in the past (e.g. Gould & Lewontin, 1979). To cite two quite different studies: birds, mammals and reptiles all use the same motion to scratch their heads: the hind limb reaches forward over the front limb. In birds this movement is both awkward and unnecessary since it means first extending the wing, and then moving the leg over it. It can only be the result of evolution from the older, more rational movement of the four-legged ancestors of birds (Lorenz, 1958). Second, in Caenorhabditis elegans, a bilaterally symmetrical nervous system is achieved through highly asymmetric development (Sulston, Schierenberg, White & Thomson, 1983). Apparently the processes which form the adult nervous system, and the behaviour produced by such a system, show such evolutionary quirks. It is therefore logical that they should also be found in the neural circuits themselves. This sort of thing should not surprise us. To quote Sydney Brenner (Lewin, 1984), 'Anything that is produced by evolution is bound to be a bit of a mess'.

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