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# ELECTRICAL AND CHEMICAL SYNAPSES BETWEEN GIANT INTERNEURONES AND GIANT FLEXOR MOTOR NEURONES OF THE HERMIT CRAB (*PAGURUS POLLICARIS*)

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## SUMMARY

1. An examination is made of the characteristics of the synapses between the single pair of giant interneurones (GIs) and the giant flexor motor neurones (GFMNs) in the fused thoracic–abdominal (TA) ganglion of the hermit crab *Pagurus pollicaris*.

2. There is an electrical synapse between each GI and its ipsilateral GFMN. Evidence for this includes (a) dye (Lucifer Yellow CH) coupling between the two neurones, (b) a short synaptic (0·2 ms) delay between spikes in the two axons, (c) the ability to pass hyperpolarizing current between the two neurones and (d) the sensitivity of the connection to bath applications of *N*-ethylmaleimide. This synaptic connection is rectifying, since a GFMN spike does not provoke an action potential in the GI.

3. There is a connection between the GI and the contralateral GFMN. Data indicating that this synaptic connection is chemical includes (a) a synaptic delay of between 0·6 and 0·8 ms, (b) transmission is easily and irreversibly fatigued, (c) the synapse is insensitive to *N*-ethylmaleimide and (d) there is no dye coupling between the two neurones.

4. Branches of the GFMN come in close proximity with the GI on both sides of the TA ganglion. However, it is not known whether there is a direct connection or an intervening neurone between the GI and the contralateral GFMN.

## INTRODUCTION

The escape response of crayfish and lobsters is composed of a rhythmic series of tail flips and is produced by alternate contractions of the (fast) deep abdominal extensor and flexor muscles. There are at least three different types of escape response. Which of the three responses is exhibited depends upon the stimulus applied and the 'command' pathway utilized (Larimer, Eggleston, Masukawa & Kennedy, 1971; Wine & Krasne, 1972). One response is produced by the medial

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giant interneurones, a second by the lateral giant interneurones and a third by a non-giant pathway (Krasne & Wine, 1977); two of these responses can be combined in one escape sequence (Reichert & Wine, 1983). At the level of the third root, the medial giant interneurones make electrically rectifying synapses with the giant flexor motor neurones on both sides (Furshpan & Potter, 1959; Mittenthal & Wine, 1978). As a result, activity in one medial giant interneurone produces an action potential in the giant flexor motor neurone on both sides.

The escape response of hermit crabs has certain characteristics similar to that of the crayfish. Stimulation of the head region elicits a rapid abdominal flexion, which quickly pulls the animal back into the gastropod shell in which it lives. This escape response is mediated by a single pair of giant interneurones (GIs), which are considered to be homologous to the medial giant interneurones of the crayfish (Wiersma, 1961; Chapple, 1966). The GIs run dorsomedially along the length of the ventral nerve cord and seem to make rectifying, electrical synaptic connections with the ipsilateral giant motor neurones to the fast abdominal flexor muscles (Umbach & Lang, 1981). The location of the GI cell body and the branching patterns in the supraoesophageal ganglion are similar to those described for the medial giant interneurone of lobster (Stephens, 1985).

Despite these similarities in the systems that control the escape response of crayfish and hermit crabs, there are differences. There is no electrical synaptic connection between the GI and the contralateral giant flexor motor neurone (GFMN), although a connection between the GI and another contralateral (presumed) flexor motor neurone has been described (Stephens, 1985). There is no lateral giant interneurone in the ventral nerve cord of the hermit crab, although there is some evidence for a non-giant pathway (P. J. Stephens, unpublished observations). Finally, on a larger scale, the layout of the nervous system is different in the two animals. In the hermit crab the thoracic ganglia are fused to form a chain and the first abdominal ganglion is fused with the fourth and fifth thoracic ganglia (Stephens, 1986) to form a single thoracic–abdominal (TA) ganglion. Since the ganglia in the abdomen are embedded in connective tissue and are less accessible, most recordings from flexor motor neurones have been done on those located in the fused TA ganglion (Umbach & Lang, 1981; Stephens, 1985).

This paper examines the type of synaptic connection that exists between the GIs and the GFMNs in the fused TA ganglion of the hermit crab. The GI on each side makes a rectifying electrical synapse with the ipsilateral GFMN and a weak, easily fatigued chemical synapse with the contralateral GFMN.

## MATERIALS AND METHODS

Male and female flat clawed hermit crabs (*Pagurus pollicaris*) were collected in the waters around Woods Hole, Massachusetts, and were held in running seawater aquaria at the Marine Biological Laboratories. The animals were fed small pieces of squid twice each week and were usually used for experimentation within 2 weeks of capture.

Preparations were made from the thoracic and fused thoracic–abdominal ganglia as described previously (Stephens, 1985, 1986). Preparations were pinned out dorsal surface uppermost in a Sylgard-lined dish and bathed in crab saline at 16°C. A mirror was placed under the dish to reflect light through the preparation so that the GI axons could be seen for glass microelectrode penetration. In many preparations the sheath over the dorsal surface of the TA ganglion was carefully removed with fine forceps prior to axon penetration with microelectrodes.

The first abdominal (1A) roots of the TA ganglion, which contain axons that innervate the flexor muscles in the last two thoracic segments (Stephens, 1986), were cut and drawn into suction recording electrodes. In most experiments brief stimulus shocks were applied to the GI in one of the oesophageal connectives. To ensure that activity was produced only in the GI, a glass microelectrode was used to impale and pass current directly into the axon (Stephens, 1985). Additional glass microelectrodes were placed in the GI and/or the GFMN in the desheathed TA ganglion; identification of the impaled axon as that of the GI or the GFMN is described in the Results section. Activity evoked by an action potential in the GI was amplified, displayed on the screen of a storage oscilloscope, and photographed with a kymograph camera using conventional techniques.

Glass microelectrodes were filled with 2 mol $l^{-1}$ potassium acetate, 4% (w/v) hexaminic cobaltous chloride or 3% Lucifer Yellow CH (w/v) in 1 mol $l^{-1}$ lithium chloride (resistance 7–40 MΩ). Penetrated axons were filled for up to 2 h with cobalt or Lucifer Yellow CH by applying 0·5-ms pulses of current (of the appropriate polarity) at a frequency of 1 Hz. The applied current (150–500 nA) was measured through a virtual ground circuit. Lucifer Yellow-injected tissue was fixed for 2 h in 4% paraformaldehyde, dehydrated through a series of alcohols, cleared in methyl salicylate, and observed and photographed through a fluorescent compound microscope. Cobalt-injected tissue was bathed in crab saline containing 10% ammonium sulphide for 15–30 min and then rinsed in fresh saline (Pitman, Tweedle & Cohen, 1972). The tissue was fixed for 2 h in 4% formalin, dehydrated and cleared as above, and then observed and photographed through a dissection microscope.

## RESULTS

The techniques used to penetrate single axons in the oesophageal connective with a microelectrode, and the criteria used to identify the impaled axon as that of the GI are described elsewhere (Stephens, 1985). Axons were also penetrated in the caudal half of the desheathed TA ganglion. In this region of the ganglion the GI axons can be easily seen on either side of the midline on the dorsal surface of the ganglion; the GFMN axon cannot be seen under the dissection microscope. The procedure used to impale GI or GFMN axons involved penetration of an axon, production of a GI action potential by electrical stimulation through the microelectrode in the oesophageal connective, and observing the response in the impaled axon and the ipsilateral 1A root (Fig. 1A). In addition, brief stimulus shocks were applied through the recording microelectrode in the GFMN or the GI to produce a response in the

1A root (Fig. 1B). Two criteria were used to determine whether the microelectrode was in the GI or the GFMN axon. First, brief stimulus shocks applied to the 1A root evoked an action potential only in GFMN (Fig. 1C). Second, injection of cobalt ions through the microelectrode and into the impaled axon revealed the morphology of the neurone. The GI axon runs without branching along the dorsal surface of the TA ganglion (Stephens, 1985), while the GFMN has its cell body on the ventral surface and has branches in the TA ganglion (Fig. 1D).

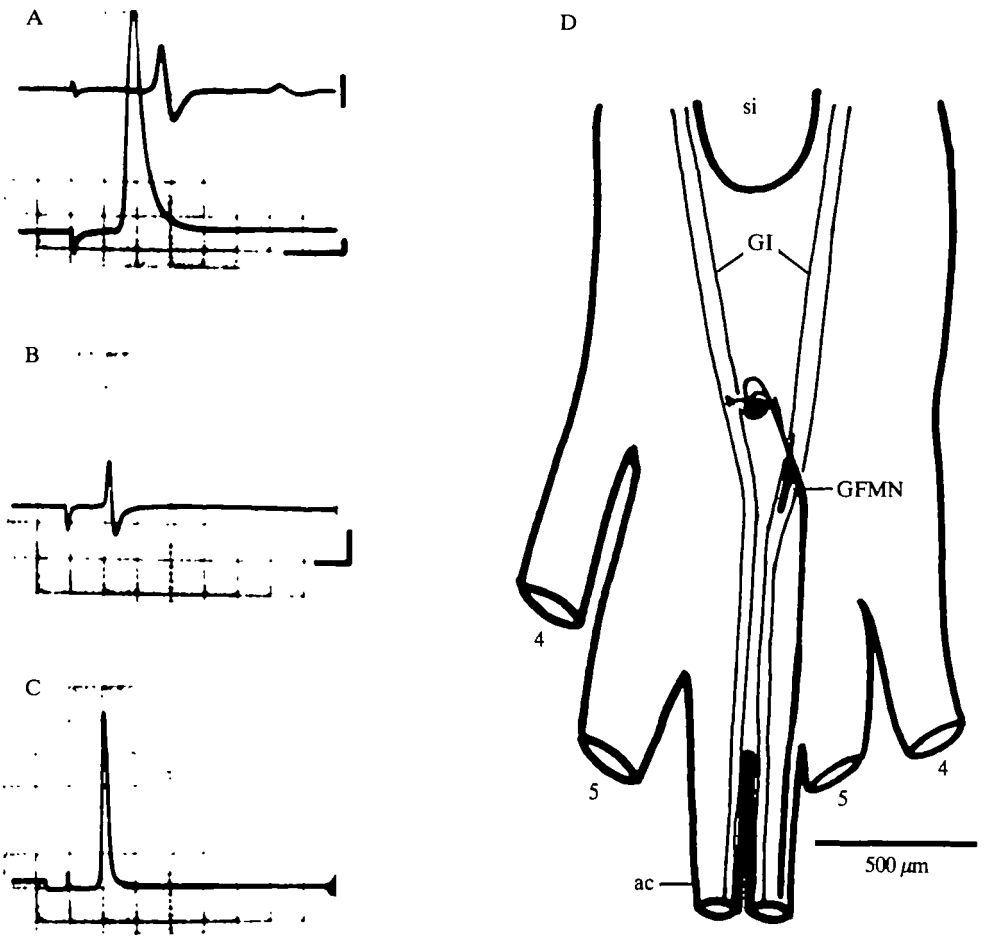


Fig. 1. Identification of a microelectrode-impaled GFMN axon in the TA ganglion. Careful grading of the stimulus shocks applied to GI in the oesophageal connective caused the synchronous appearance of a GFMN spike in the ipsilateral 1A root (upper trace) and a spike (lower trace) in the impaled axon (A). Stimulation through the microelectrode evoked a GFMN spike in the 1A root (B), and stimulation of the 1A root produced a spike in the GFMN axon (C). A *camera lucida* drawing of a fixed TA ganglion preparation in which the right GFMN was injected with cobalt (through the microelectrode) (D). Notice the overlap of the GFMN with the GI on each side. The numbers represent the thoracic roots; ac, the abdominal connectives; si, the sinus. Scale bar, 5 mV (intracellular), 500 $\mu$V (extracellular) and 2 ms; 500 $\mu$m (D).

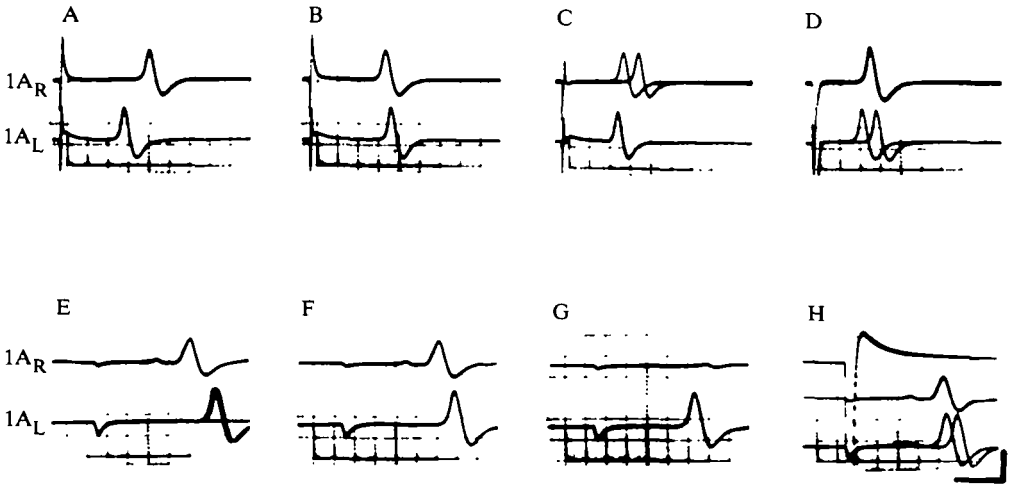


Fig. 2. GFMN responses recorded from the right (R) and left (L) 1A roots in response to stimulation of the GIs in the oesophageal connectives. Stimulation of the left GI (A), both GIs (B) and the traces superimposed (C); traces are superimposed for stimulation of the right GI and the both GIs (D). Note that the response is delayed by about 0·8 ms when the contralateral GI is stimulated. Five shocks applied to the right GI (at 10 Hz) produced fluctuating delays and a failure of the left GFMN (E); stimulation of the right GI produced activity in both GFMNs (F) even though the connection between the left GI and the right GFMN was fatigued (G). The responses (superimposed) recorded following stimulation of the right GI and then both GIs (H). Note (1) the delayed response in the left GI when only the right GI was stimulated and (2) no activity was recorded from the left GI in the oesophageal connective (upper trace) – the response produced when both GIs were stimulated is lost in the stimulus artifact. Scale bar, 500 $\mu$V and 2 ms.

### *GIs connection with the contralateral GFMN*

When brief stimulus shocks were applied to the GI axon on the left or the right side, activity was evoked in the GFMNs on both sides (Fig. 2). These results were obtained when the GI axon was stimulated in the oesophageal or in the abdominal connective, using stimulus shocks applied either through a microelectrode in the axon or *via* hook electrodes placed under the connective. The time delay between GI axon stimulation and the GFMN response depended upon which GI was stimulated. This became obvious when the response produced by stimulation of one GI was superimposed upon the response recorded when both GIs were stimulated (Fig. 2C,D). The delay of GFMN activity was 0·6–0·8 ms longer when produced by stimulation of the contralateral GI.

In all preparations the synaptic connection between the GI axon and the contralateral GFMN could be quickly fatigued by maintained repetitive GI stimulation. During trains of GI shocks the response evoked in the contralateral GFMN usually exhibited fluctuating delays and failures (Fig. 2E). Maintained repetitive stimulation of the GI axon resulted in irreversible fatigue and failure of the connection to the contralateral GFMN. In these fatigued preparations, however, the connection between the (stimulated) GI and its ipsilateral GFMN was still intact, as was

the connection between the GI on the other side and its contralateral GFMN (Fig. 2F,G).

When stimulation of a GI axon produced activity in the contralateral GFMN, no activity was recorded from the contralateral GI axon (Figs 2H, 3A,B). When the connection between the GI axon and the contralateral GFMN was fatigued, intracellular recordings made from GFMN axon in the TA ganglion showed a small depolarizing potential in response to a GI axon spike (Fig. 3C). High-frequency stimulation of the GI axon produced a little summation, but no facilitation (Fig. 3D).

*GIs connection with the ipsilateral GFMN*

To test whether there is an electrical connection between the GI and the ipsilateral GFMN (Umbach & Lang, 1981), preparations were exposed to *N*-ethylmaleimide (NEM) (2–10 mmol $l^{-1}$ in crab saline), an agent known to uncouple electrical synapses (Spray *et al.* 1984). Fig. 4A shows the response recorded from the GFMNs on both sides to stimulation of the left GI axon. Application of NEM produced a spontaneous and prolonged (2–5 min) spike discharge in axons in the 1A roots (Fig. 4B) and the abdominal connectives. Bathing preparations in 5 mmol $l^{-1}$ NEM caused the GI axon to depolarize by 10–15 mV and resulted in a decline and

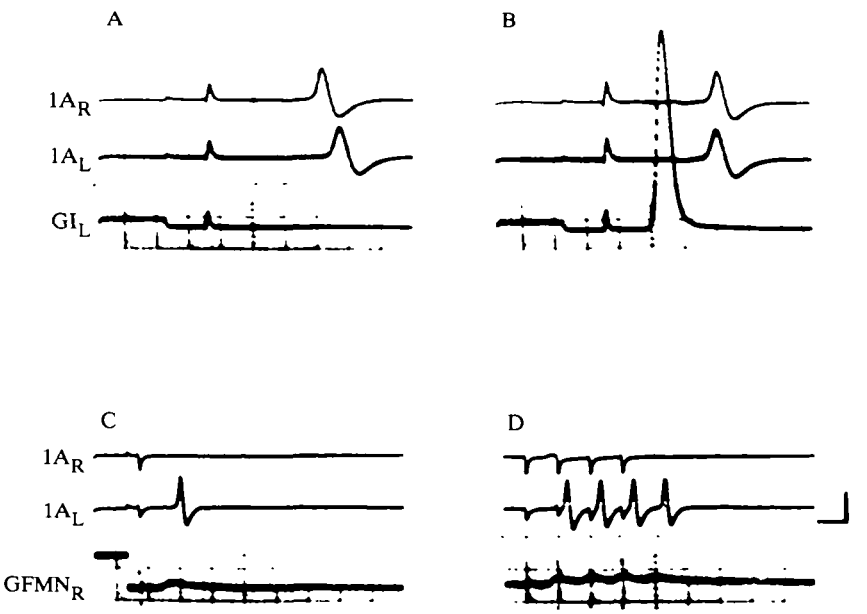


Fig. 3. GFMN responses recorded from the right (R) and left (L) 1A roots and the left GI in response to stimulation of the right GI (A) and the left GI (B) in the oesophageal connective. Note that stimulation of the right GI produced activity in the left GFMN but not in the left GI. In a fatigued preparation, left GI stimulation produced a small amplitude depolarization in the right (R) GFMN (C) which exhibited a degree of summation at high frequencies (D). Scale bar, 5 mV (intracellular), 500 $\mu$V (extracellular) and 2 ms.

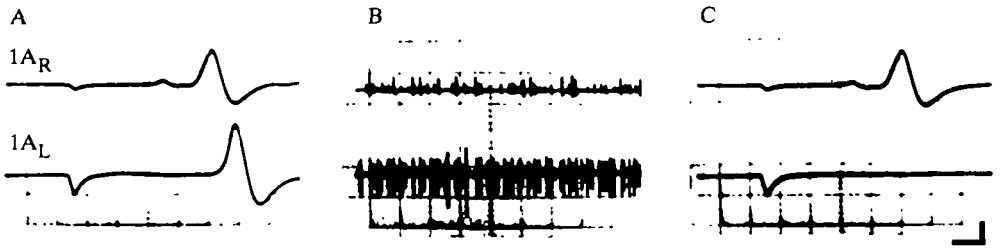


Fig. 4. The effect of *N*-ethylmaleimide (NEM) on the GFMN responses produced by a spike in the left GI. In normal saline, activity was produced in both GFMNs (A). Application of 5 mmol $l^{-1}$ NEM evoked spontaneous activity in the 1A roots (B); a spike in the left GI produced activity only in the right GFMN. Scale bar, 200 $\mu$V, 1 ms (A,C) and 100 ms (B).

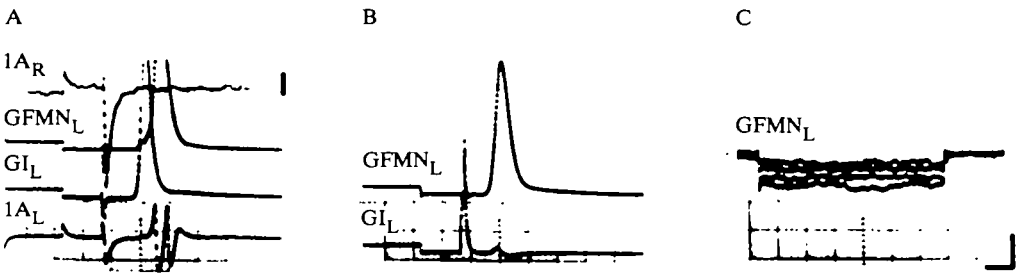


Fig. 5. Activity recorded from the left (L) and right (R) 1A roots and the left GFMN and GI (intracellularly in the TA ganglion) in response to stimulation of the left GI in the oesophageal connective (A). The suction electrode on the left 1A root was very close to the abdominal connective so the second response probably represents the left GI spike. Stimulation of the left GFMN in the 1A root produced a spike in the GFMN but only a small depolarization in the left GI (B). Hyperpolarizing current injected into the left GI could be recorded from the left GFMN (C); this coupling was abolished by 5 mmol $l^{-1}$ NEM. Scale bar, 5 mV (intracellular), 500 $\mu$V (extracellular), 2 ms (A,B) and 50 ms (C).

then failure of the action potential – paradoxically, no spontaneous spike activity was recorded from the GI or the GFMN during this time. If the GI axon was stimulated before spike failure, activity was recorded from the contralateral (not the ipsilateral) GFMN (Fig. 4C). At this stage in these preparations, brief stimulus pulses applied directly to the 1A root demonstrated that spikes could be produced in the ipsilateral GFMN. Thus the GI and GFMN axons could still produce action potentials, indicating that the synaptic connection between the two neurones was affected by NEM.

In certain preparations, glass microelectrodes were simultaneously placed into the GI and the GFMN axons in the TA ganglion. In Fig. 5A stimulation of the left GI in the oesophageal ganglion provoked spikes in the left GI axon and the left GFMN axon. Since the location of the synaptic connection between these two cells is not known, it was not possible to measure the distance between the electrode sites and the synapse. However, the time delay between the spikes in the two axons (about 0·2 ms) is too short to be produced by transmission across a chemical synapse.

When brief stimulus pulses were applied to the left 1A root, a spike was recorded from the left GFMN in the TA ganglion and soon thereafter a small depolarization in

the GI axon (Fig. 5B). The synaptic connection between the GI and GFMN is, therefore, rectifying. Hyperpolarizing current pulses applied through the microelectrode in the GI axon hyperpolarized the GFMN (Fig. 5C). This coupling was abolished by the application of 5 mmol $l^{-1}$ NEM.

When an axon in the TA ganglion was impaled with a glass microelectrode, it was possible to inject 'dye' iontophoretically. When Lucifer Yellow CH was injected into the GI axon, other neurones were also filled with the 'dye'. One example can be seen in Fig. 6A. The cell body is located in the midline on the ventral surface and sends its axons dorsally and then caudally. Similar observations have been made in preparations in which the GFMN was impaled with a microelectrode and filled with cobalt (Fig. 6B). These results indicate that the neurone that is dye coupled to the GI axon in the TA ganglion may be the ipsilateral GFMN. This would support the contention that these two cells are linked by an electrical synapse (Stewart, 1978; Umbach & Lang, 1981). Finally, wholemount preparations of TA ganglia in which the GFMN was filled with cobalt show that the motor neurone has branches that lie in close proximity to the ipsilateral and contralateral GI axons (Fig. 1D).

## DISCUSSION

Data reported in this paper demonstrate that in the fused TA ganglion of the hermit crab the GIs make functional synaptic connections with the GFMNs on both sides (Fig. 2). In the abdominal ganglia of crayfish and lobster, there are rectifying electrical synapses between the medial giants and the GFMNs on both sides (Furshpan & Potter, 1959; Mittenthal & Wine, 1978). In the hermit crab the connection between the GI and the ipsilateral GFMN appears to have the same characteristics. Transmission between the GI and GFMN is unidirectional and the synaptic delay is short (Fig. 5) – too short for transmission across a chemical synapse (Hagiwara & Tasaki, 1958). Hyperpolarizing current can be transmitted between the two neurones, indicating that the cells are electrically coupled. Further, Lucifer Yellow CH is known to travel across electrical junctions (Stewart, 1978). When the GI was injected with Lucifer Yellow CH there was dye coupling with a neurone in the TA ganglion (Fig. 6A) whose morphology closely resembled that of the GFMN (Fig. 6B). If this dye-coupled cell is the GFMN, these results would be consistent with the dye coupling seen between the GI and GFMN in crayfish abdominal ganglia (Margiotta & Walcott, 1983). Finally, the connection between the GI and the ipsilateral GFMN in the TA ganglion can be uncoupled by bath application of NEM, which is a potent uncoupler of electrical synapses (Spray *et al.* 1984). These data are consistent with the idea that the connection between the GI and the ipsilateral GFMN is a rectifying electrical synapse.

In the fused TA ganglion of the hermit crab there is a functional connection between the GI and the contralateral GFMN (Fig. 2). This observation is in contrast to previous work (Umbach & Lang, 1981; Stephens, 1985) in which only GI's connection with the ipsilateral GFMN was reported; I have reported a connection between the GI and a motor neurone with an axon in the contralateral 1A

root (Stephens, 1985). The reason why the connection between the GI and the contralateral GFMN has not been reported previously may be that the synapse is weak and is quickly fatigued. A second, easily fatigued synapse has been described between the two GIs in the brain (Stephens, 1985). The properties of the synapse

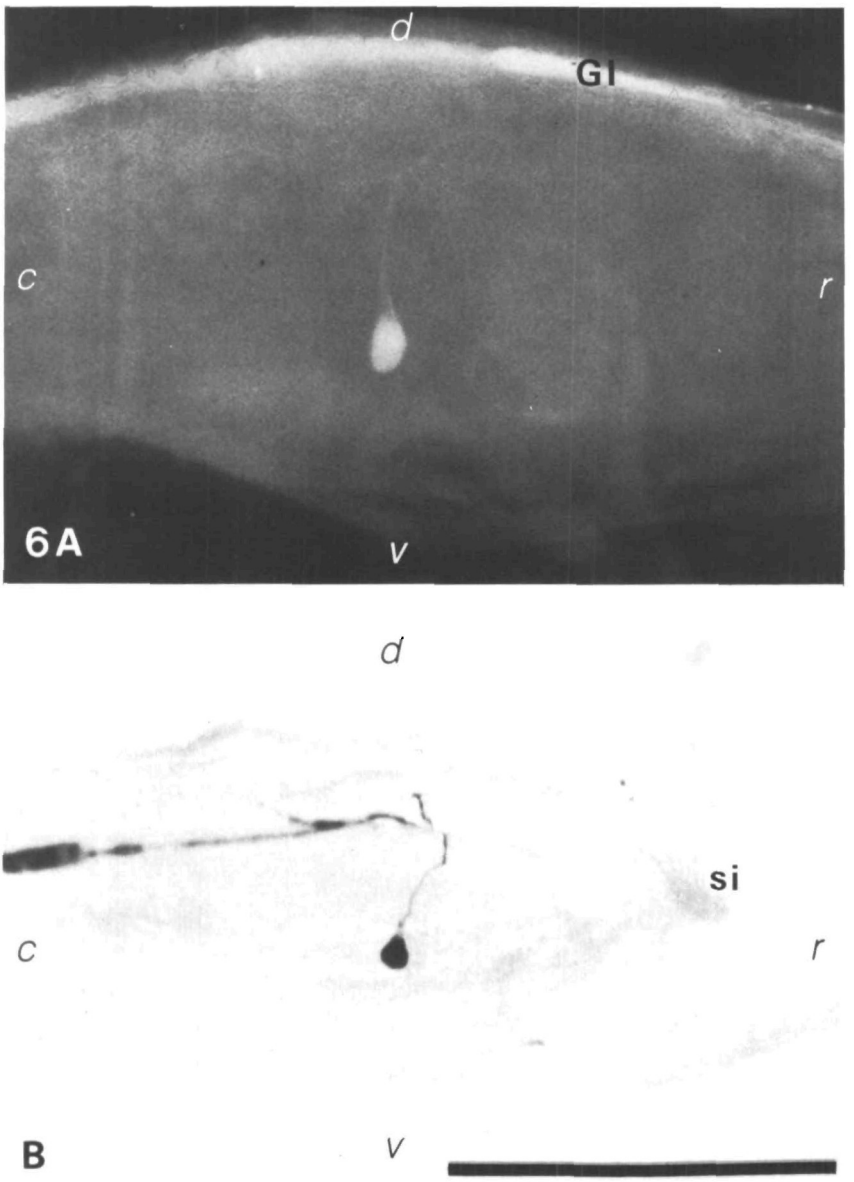


Fig. 6. Lucifer Yellow CH injected into right GI axon in the TA ganglion (A) revealed dye coupling with a cell that has a similar morphology to the right GFMN; stained intracellularly in a second preparation with cobalt (B); the views are lateral and are taken from the right side. *c*, caudal; *d*, dorsal; *r*, rostral; si, sinus; *v*, ventral. Scale bar, 400 μm (A) and 500 μm (B).

from the GI to the contralateral GFMN are different from the connection to the ipsilateral GFMN. The connection shows irreversible fatigue – a characteristic that has never been observed for the synapse to the ipsilateral GFMN (P. J. Stephens, unpublished observations). Immediately prior to synaptic fatigue the stimulus–response delay fluctuates (Fig. 2E). The GFMN spike has a delay 0·6–0·8 ms longer when produced by stimulation of the contralateral GI as opposed to *via* the electrical synapse with the ipsilateral GI (Fig. 2). Since the conduction velocities of pairs of GI axons are similar (Stephens, 1985), this difference in delay must be due to the time taken for spike conduction and synaptic transmission. The value for the increased response delay is about the same as that reported for other chemical synapses (Hagiwara & Tasaki, 1958), including the synapse between the two GIs in the supraoesophageal ganglion (Stephens, 1985). Finally, NEM uncouples the electrical synapse between the GI and its ipsilateral GFMN, but has no effect on GI's synaptic connection with its contralateral GFMN (Fig. 4). If the connection between the GI and its contralateral GFMN is a chemical synapse, the small depolarization recorded from the contralateral GFMN in fatigued preparations (Fig. 3C,D) may be a postsynaptic potential which is sub-threshold for spike production.

The above data would indicate that the synapse between the GI and the contralateral GFMN is chemical. It seems possible that this connection could be made in at least *three* ways: (1) through the contralateral GI, (2) directly from the GI to the contralateral GFMN, or (3) *via* a third intervening neurone. The first possibility can be ruled out since activity need be present only in one GI to provoke activity in both GFMNs (Figs 2H, 3A,B). The second possibility seems likely since the GFMN has branches that come close to both GI axons in the TA ganglion (Fig. 1D). However, there is no evidence that rules out the possibility that there is a neurone between the GI and the contralateral GFMN.

Irrespective of the nature of the synaptic connections between the GIs and the GFMNs, the question remains as to the functional significance of these connections to the hermit crab's behaviour. The GI–GFMN pathway is used to produce a rapid escape, to withdraw the animal back into the gastropod shell in which it lives. It seems reasonable that the most effective way to perform this function would be to use the fast flexor muscles on both sides. No work has been reported to date on the effects of different sensory inputs on the activity of one or both GIs. However, in the supraoesophageal ganglion there is a bidirectional synapse between the two GIs, which ensures that a spike in one GI produces an action potential in the other (Stephens, 1985). There are connections between the GIs and the contralateral GFMNs (Fig. 2). Therefore, it appears that if there is a spike in one GI, activity can be produced in the contralateral GFMN either *via* a synapse with the contralateral GI in the brain or *via* a synapse with the contralateral GFMN in the TA ganglion. However, these synapses show rapid fatigue *in vitro* (Fig. 2; Stephens, 1985) and may act as safety factors for one another. This must be confirmed by recording from the GIs and GFMNs *in vivo*. Finally, the escape response is further complicated by the connection between the GI and a second contralateral motor neurone (Stephens,

1985), which may innervate the flexor muscles, and a second ipsilateral excitatory motor neurone which innervates the ventral (slow) flexor muscle (Stephens, 1986). The escape response is very different from that described in crayfish, where the GI inhibits the slow neuromuscular system (Kuwada & Wine, 1979). Further work recording from intact animals is presently in progress to determine the activity of the different muscles during the hermit crab escape response.

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## REFERENCES

CHAPPLE, W. D. (1966). Asymmetry of the motor system in the hermit crab *Pagurus granosimanus* Stimpson. *J. exp. Biol.* **45**, 65–81.

FURSHPAN, E. J. & POTTER, D. D. (1959). Transmission at the giant motor synapses of the crayfish. *J. Physiol., Lond.* **145**, 289–325.

HAGIWARA, S. & TASAKI, I. (1958). A study on the mechanism of impulse transmission across the giant synapse of the squid. *J. Physiol., Lond.* **143**, 114–137.

KRASNE, F. B. & WINE, J. J. (1977). Control of crayfish escape behavior. In *Identified Neurons and Behavior of Arthropods* (ed. G. Hoyle), pp. 275–292. New York: Plenum Press.

KUWADA, J. Y. & WINE, J. J. (1979). Crayfish escape behavior: commands for fast movement inhibit postural tone and reflexes, and prevent habituation of slow reflexes. *J. exp. Biol.* **79**, 205–224.

LARIMER, J. L., EGGLESTON, A. C., MASUKAWA, L. M. & KENNEDY, D. (1971). The different connections and motor outputs of lateral and medial giant fibres in the crayfish. *J. exp. Biol.* **54**, 391–402.

MARGIOTTA, J. F. & WALCOTT, B. (1983). Conductance and dye permeability of a rectifying electrical synapse. *Nature, Lond.* **305**, 52–55.

MITTENTHAL, J. E. & WINE, J. J. (1978). Segmental homology and variation in flexor motoneurons of the crayfish abdomen. *J. comp. Neurol.* **177**, 311–334.

PITMAN, R. M., TWEEDLE, C. D. & COHEN, M. J. (1972). Branching of central neurones: Intracellular cobalt injection for light and electron microscopy. *Science* **162**, 281–283.

REICHERT, H. & WINE, J. J. (1983). Coordination of lateral giant and non-giant systems in crayfish escape behavior. *J. comp. Physiol.* **153**, 3–15.

SPRAY, D. C., WHITE, R. L., CAMPOS DE CARVALHO, A., HARRIS, A. L. & BENNETT, M. V. L. (1984). Gating of gap junction channels. *Biophys. J.* **45**, 219–230.

STEPHENS, P. J. (1985). Morphology and physiology of the giant interneurons of the hermit crab (*Pagurus pollicaris*). *J. Neurobiol.* **16**, 361–372.

STEPHENS, P. J. (1986). The fused thoracic–abdominal ganglion of the hermit crab (*Pagurus pollicaris*): neuromuscular relationships in the thoracic and abdominal flexor muscles. *J. exp. Biol.* **123**, 201–216.

STEWART, W. W. (1978). Functional connections between cells as revealed by dye-coupling with a highly fluorescent napththalimide tracer. *Cell* **14**, 741–759.

UMBACH, J. A. & LANG, F. (1981). Synaptic interaction between the giant interneuron and the giant motorneuron in the hermit crab *Pagurus pollicaris*. *Comp. Biochem. Physiol.* **68**A, 49–53.

WIERSMA, C. A. G. (1961). Reflexes and the central nervous system. In *The Physiology of Crustacea*, vol. II (ed. T. H. Waterman), pp. 241–297. New York: Academic Press.

WINE, J. J. & KRASNE, F. B. (1972). The organization of escape behavior in the crayfish. *J. exp. Biol.* **56**, 1–18.