

## COORDINATED EXCITATION OF FLEXOR INHIBITORS IN THE CRAYFISH

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(Received 8 August 1979)

### SUMMARY

In isolated abdominal nerve cords of crayfish, the medial or lateral giant axons were stimulated at a position just rostral to the first abdominal ganglion. Recordings of the impulse sequences of the flexor inhibitor (FI) were made from the anterior five ganglia, three ganglia at a time.

In 20% of our preparations, one giant axon impulse caused one to four FI impulses in every abdominal third root. An equal number of FI impulses were usually produced by each abdominal ganglion for any given stimulation.

The earliest FI impulse was observed at the third root of the fourth ganglion. FI impulses occurred with increasing latencies rostrally and caudally from the fourth ganglion.

The FI responses to medial and lateral giant axons stimulation were essentially equivalent.

FI impulses were recorded from the rostral three abdominal ganglia, while the caudal ganglia were cut off one after another from the sixth to the third ganglion. Little change was noted until after the removal of the fourth ganglion, which usually caused all FI impulses to disappear.

From these experimental results, we propose a model of central mechanisms for FI excitation.

### INTRODUCTION

The abdominal flexion of crayfish is activated by three neural systems (Wine & Krasne, 1972): the medial giant, lateral giant and non giant fibre systems. The quickest escape response is triggered by the giant fibres. The motor neurones causing this response innervate the fast flexor muscles through abdominal third roots (Selverston & Remler, 1972). Abdominal third roots have 7-11 motor axons, one of which is the inhibitor (Mittenthal & Wine, 1978). In order to understand the coordination of the abdominal muscle contraction, it is important to measure not only the impulse sequences of the individual nerves, but also differences in timing between the ganglia. In this report, we describe the inter-ganglionic differences of flexor inhibitor (FI) responses recorded from the isolated abdominal nerve cord.

## ABDOMINAL NERVE NETWORK

The abdominal nervous system of the crayfish consists of six ganglia. The rostral five ganglia have three pairs of nerve roots. The third root which innervates the fast abdominal flexors branches out from the ventral cord at a position slightly caudal to the ganglion. The third root bifurcates within 1 mm from the point at which it branches from the ventral nerve cord; one of the third root branches extends laterally and the other dorsally to the muscles (Kennedy & Takeda, 1965; Rayner & Wiersma, 1967). Only one of the axons in the third root is inhibitory. Selverston & Remler (1972) investigated the soma position and peripheral destination of the ten axons of the third ganglion. Mittenthal & Wine (1978) described that the somata of the peripheral inhibitor (FI) in the anterior five abdominal ganglia extend their axon from the contralateral third root of the same ganglion to flexor muscles.

## METHODS

Crayfish (*Procambarus clarkii*) were cooled at 10 °C for about 1 h to avoid nerve deterioration during the experiments, which were carried out at 22 °C. Recordings were made from the third roots of ganglia in an isolated nerve cord, as follows. A crayfish was pinned ventral side in a dissecting dish. The superficial exoskeleton was carefully removed from segments 1–6, exposing the flexor muscles and the ventral nerve cord. The first and the second roots of all abdominal ganglia were cut. The third roots were cut as close to the muscle as possible to obtain as much axon as possible. The chain of six ganglia was then dissected out and fastened in a lucite vessel filled with Van Harreveld solution (1936). The third roots were lifted with hook-shaped platinum electrodes for recordings. Signals were amplified with IC operational amplifiers, recorded on 4-channel tape and displayed on an oscilloscope. Tapes were played back at slow speed on to a chart recorder for analysis.

Giant axons were stimulated using an electrode made from a glass tube drawn to a tip diameter of about 60  $\mu\text{m}$ . The surface was covered with evaporated gold for the anode, and a platinum wire embedded inside for the cathode. The tip diameter of the stimulation electrode was smaller than the diameter of the medial giant (MG) or lateral giant (LG) axons. Stimulation was made on the rostral part of a medial or lateral axon of the first ganglion. The following abbreviations are used in this paper; S:L(R)M and S:L(R)L denote the stimulus on the left(right) medial giant axon and the stimulus on the left(right) lateral giant axon, respectively.

In one series of experiments, abdominal flexion was filmed at 200 or 400 frames/s. The side of the animal was held against a plate, by a rubber band around the cephalothorax, to keep the animal in the plane of focus. Flexion was triggered by stimulation of the MG or LG in the circumoesophageal connective.

## RESULTS

FI impulses were recorded from 20 preparations. No impulses were recorded from 80 other preparations, presumably as a result of the isolation procedure.

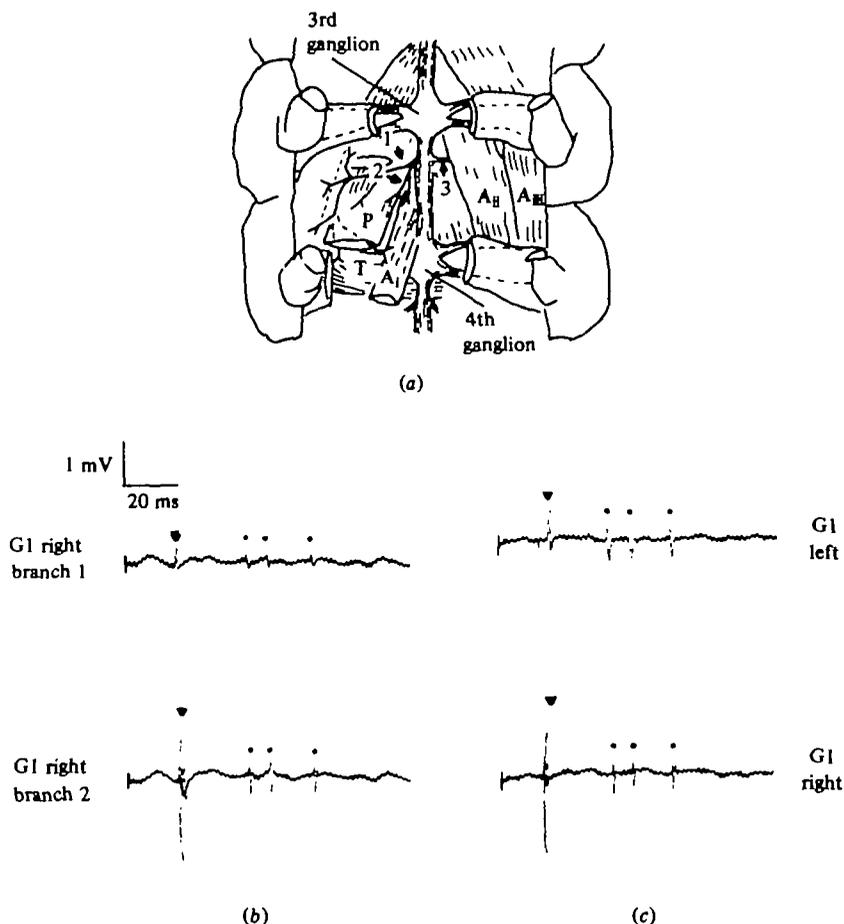


Fig. 1. (a) Ventral dissection of the third ganglion to show the first two forks (1+2) of the right third root and left third root (3).  $A_i$ ,  $A_{ii}$ ,  $A_{iii}$ ,  $P$  and  $T$  are the fast flexor muscles (Kennedy & Takeda, 1965; Rayner & Wiersma, 1976). (b) Recordings from the first two forks of the third root of the first ganglion in an isolated preparation. Upper trace from a site corresponding to arrow 1 in (a); lower trace from a site corresponding to arrow 2. (c) Recordings from the left third root (upper trace; corresponding to arrow 1 in (a)) of the same preparation as for (b). The compound pulse (▼) was recorded from the GMF and excitatory axons. The dots (●) show FI impulses.

### Identification of FI impulses

To identify the FI impulses, recordings were made from the two branches of the first bifurcation of a third root (at equidistant points from the bifurcation), since according to Furshpan & Potter (1959) and Selverston & Remler (1972), only the MoG and the FI branch at this bifurcation. A 1:1 relationship was found for up to 4 impulses per stimulus pulse (Fig. 1*b*), consistent with the results of Roberts (1968*b*) and Wine & Mistick (1977), and these impulses are deduced to be those of the FI.

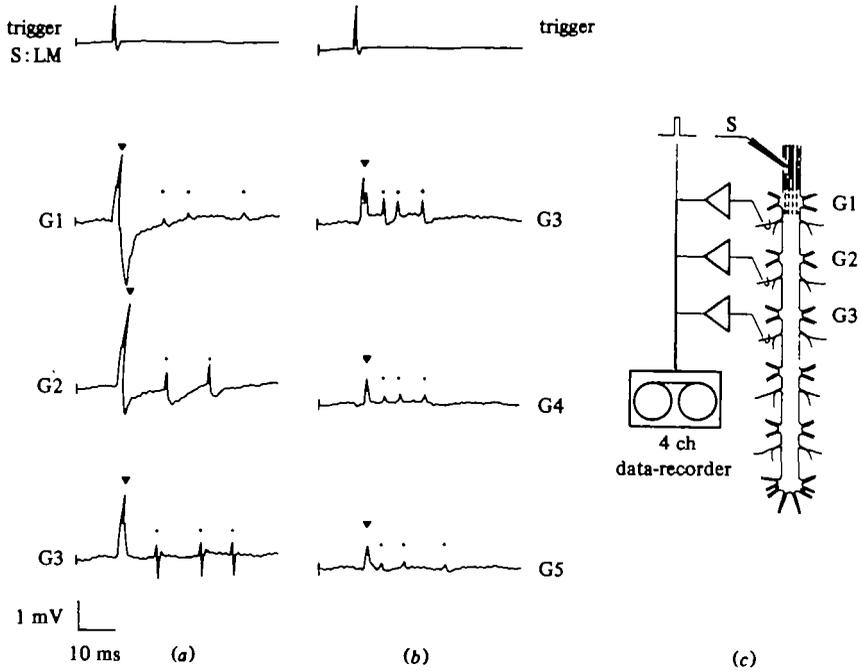


Fig. 2. Recordings from abdominal roots, made simultaneously at (a) ganglia 1-3 (G1-3), (b) ganglia 3-5 (G3-5). Recording arrangement for (a) is shown in (c). Trigger: timing of stimulating pulse. S: stimulating electrode. Symbols ( $\blacktriangledown$  and  $\bullet$ ) as Fig. 1.

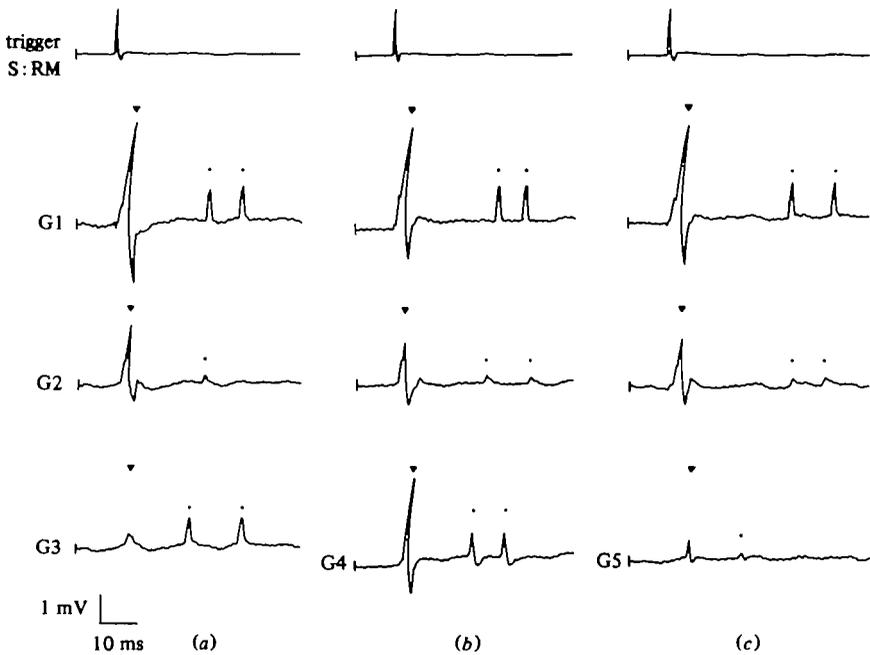


Fig. 3. Recordings showing that excitation of FIs is earlier in the caudal direction. Comparison of impulses of ganglia 1 and 2 (G1 and G2) with impulses in (a) G3; (b) G4; (c) G5. Symbols ( $\blacktriangledown$  and  $\bullet$ ) as Fig. 1.

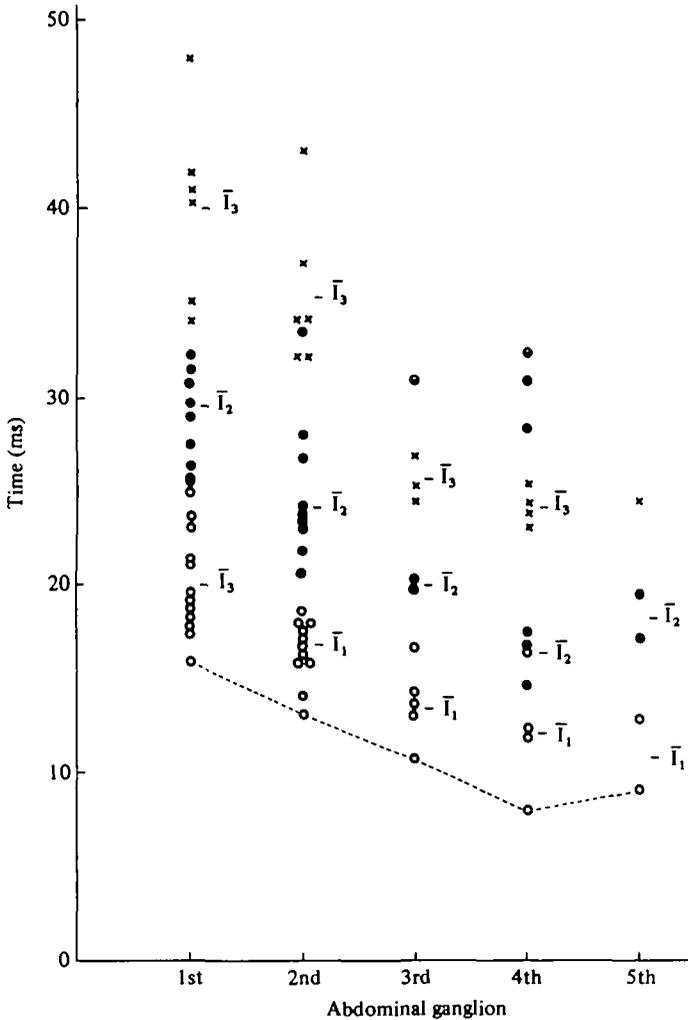


Fig. 4. Timing of FI impulses in ganglia 1-5. Each point represents the mean time after stimulation that was obtained with each arrangement of stimulating and recording electrodes for the first (○), second (●), third (×) and fourth (⊙) impulses. The records were obtained from five crayfish, from both sides where possible. The dashed line connects data from one crayfish.  $I_i$  indicates the mean timing of the  $i$ th impulse, where  $i = 1-3$ .

*Symmetry of the FI response*

The FI response was found to be essentially identical in left and right third roots of the same ganglia (Fig. 1c), indicating that the FI axons on the two sides of a ganglion share a common input mechanism. It was therefore considered valid to apply a stimulus pulse at only one giant axon for the experiments below.

*Comparison of FI responses in different ganglia*

It was possible to record from no more than three ganglia at once.

The number of FI impulses elicited in each ganglion per stimulus pulse was

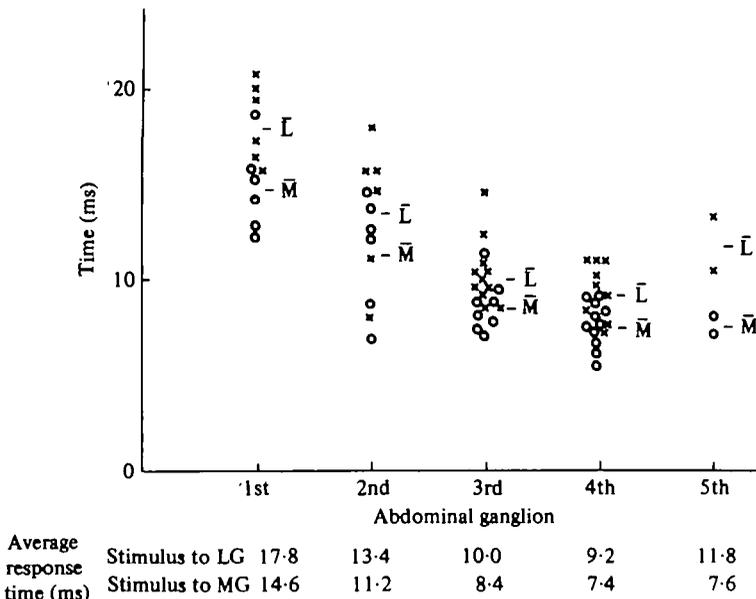


Fig. 5. Dependence of timing of FI impulses on whether stimulation was to the MG (O) or LG (x). Each point represents the mean timing of the first FI impulses after stimulation. The records were obtained from ten crayfish, from both sides where possible.

compared in recordings made from ganglia 1-3 simultaneously, and from ganglion 3-5 simultaneously. A similar number of FI impulses (1-4) was observed with each comparison (Fig. 2).

FI impulses occurred earlier in the caudal ganglia than in the rostral ones in recordings made simultaneously from the first two ganglia and the third (Fig. 3a), or the fourth (Fig. 3b), or the fifth (Fig. 3c). This was further investigated by averaging the time of occurrence of FI impulses. The mean data obtained with each arrangement of stimulating and recording electrodes (Fig. 4) showed that the first FI impulse was obtained earlier in the fourth ganglion than in the others, as seen in the data points joined by the dashed line. Thus the excitation causing the FI impulses seemed to propagate from the fourth ganglion, both rostrally and caudally. Grand means ( $\bar{I}$  in Fig. 4) are misleading because the number of impulses recorded at each ganglion was not constant. Thus, impulses were often absent in recordings from the fifth ganglion, and more impulses were recorded from the third and fourth ganglia than the others.

#### *Influence of MG and LG*

As noted in Methods, stimulation was delivered to either the MG or the LG. This can have little effect on the results, for the mean timing of the first FI impulse at each ganglion was only slightly earlier, 2.6 ms on an average, with MG rather than LG stimulation (Fig. 5). The difference is consistent with slower propagation of the trigger pulse in the LG, which has a septum in each ganglion.

There was no difference between results obtained with ipsilateral and contralateral stimulation (results not shown).

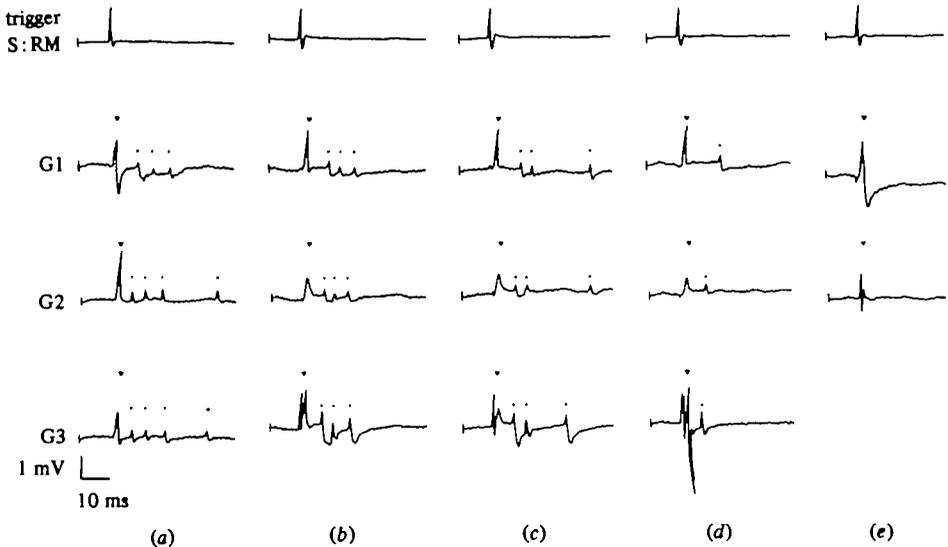


Fig. 6. FI impulses recorded in rostral ganglia, before removal of caudal ganglia (a); after removal of G6 (b); G5 (c); G4 (d); and G3 (e). The greatest effect was caused by removal of the fourth ganglion. Symbols (▼ and ●) as Fig. 1.

#### Importance of the fourth ganglion

The importance of the fourth ganglion in central excitation of the FI was investigated further by stepwise removal of the caudal ganglia while recording from ganglia 1–3 simultaneously, in five preparations.

The timing of FI impulses observed before removal of ganglion (Fig. 6a) was unaffected by removal of the sixth ganglion (Fig. 6b). Removal of the fifth ganglion sometimes had no effect (Fig. 6c), but generally the frequency of the impulses tended to increase (data not shown). Therefore the fifth ganglion may exert some inhibitory influences on FI excitation. Removal of the fourth ganglion abolished the FI response in some preparations (data not shown) and resulted in one or two impulses in others (Fig. 6d). After the third ganglion was cut, no FI impulses were observed (Fig. 6e).

In further experiments, stimulation was applied to the MG just rostral to isolated ganglion. FI impulses were observed in the third root of the isolated fourth ganglion but not of the other ganglia.

#### The timing of abdominal flexion

Film of the abdominal flexion following stimulation of the MG in the circumoesophageal connective showed that flexion began earliest at the third segment, with a mean delay of 14.2 ms. Flexion began in the first segment at 23.7 ms, in the second segment at 17.3 ms, in the fourth segment at 17.1 ms, and in the fifth segment at 19.1 ms. In the sixth segment, the starting time of the flexion was not measured. LG stimulation caused a slightly later response in the fourth and fifth segments, at 20.5 and 26.4 ms, respectively. Flexion was completed 70–80 ms after stimulation. This is greater than the value of 28 ms obtained by Roberts (1968a) and 50 ms observed by Larimer *et al.* (1971), possibly because our crayfish were tied to a plate.



FI excitation commences after excitation of the other axons in the third root, but appears to precede flexion of the abdomen, in each segment. In the fourth segment, for example, FI excitation commenced at around 11 ms after stimulation (in isolated nerve cords) whereas flexion began after about 17 ms (in the whole animal). Further experiments are required to describe the role of the FI in abdominal flexion.

We thank Professor B. Kondo for suggestions and discussion.

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