

THORACIC LEG CONTROL OF ABDOMINAL EXTENSION IN THE CRAYFISH, *PROCAMBARUS CLARKII*

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SUMMARY

Postural extensions of the abdomen of the crayfish, *Procambarus clarkii*, could be evoked by mechanical stimulation of a single thoracic leg. Movement of a single leg joint was sufficient to initiate an extension response. Vigorous abdominal extensions were initiated either by depression of the whole leg (WLD) or by flexion of the mero-carpal joint (MCF). Weaker extension responses were obtained by depression of the thoracic-coxal and coxo-basal joints. Similar stimulation of the chelipeds did not elicit an abdominal extension response.

Single-frame analysis of motion pictures of crayfish responding to WLD or MCF stimulation of a 2nd thoracic leg showed that the responses evoked by the two different stimulus situations were nearly identical. They differed principally in the responses of the leg located contralateral to the stimulated leg.

Movements of most of the cephalic, thoracic and abdominal appendages accompanied the abdominal extension response. Only the eyes remained stationary throughout the response. The mean values of the latencies for the initiation of appendage movement ranged from 125 to 204 ms; abdominal movement had a mean latency of about 220 ms.

The abdominal extension reflex resulted from the activity of the tonic superficial extensor muscles. The deep phasic extensor muscles were silent during the response. The mean latencies for the initiation of superficial extensor muscle activity by WLD and MCF stimulation were 53.7 and 50.0 ms respectively.

INTRODUCTION

Control of abdominal movement is important for the successful execution of behaviour patterns of many decapod crustaceans (crayfish, lobster, spiny lobster, hermit crabs, etc.). Such patterns are involved in escape, control of equilibrium, locomotion, reproduction and defence. In many instances the responses can be evoked by specific sensory stimuli. For example, visual stimuli may elicit defence (Glantz, 1974), walking (Davis & Ayers, 1972) or escape responses (Wine & Krasne, 1972); tactile stimuli can trigger escape (Wine & Krasne, 1972), defence (Tsukada, 1974) or swimming (Wine & Krasne, 1972); and proprioceptive stimuli may produce equilibrium reactions (Davis, 1968; Larimer & Eggleston, 1971; Page, 1975*b*) Schone *et al.* 1976).

In each of these behaviour patterns the segmented abdomen undergoes a characteristic sequence of movements produced by contractions of the abdominal musculature. The abdominal muscles of decapods are divided into two divisions; the deep phasic extensors and flexors, and the superficial tonic extensors and flexors. The phasic musculature is activated during rapid abdominal movements such as those which generate escape or swimming responses (Kennedy & Takeda, 1965*a*; Wine, 1977). Changes in abdominal posture result from contractions of the tonic musculature (Kennedy & Takeda, 1965*b*).

The neuronal systems which control the posture of the crayfish abdomen comprise one of the 'classic' preparations for investigation of the cellular bases of behaviour (Kennedy & Davis, 1977; Page, 1980). Equilibrium responses, which include postural extensions of the abdomen, can be evoked in crayfish (*Procambarus clarkii*) by the loss of contact between the legs and a supporting substrate. The stimulus situations used to elicit these extension responses include removal of a platform supporting the legs (Larimer & Eggleston, 1971) or lifting an animal up from the substrate into the water column (Page, 1975*b*). Sensory receptors that could be involved in the mediation of these extension reflex responses include statocysts, leg proprioceptors and contact receptors, receptors sensitive to vibrations and water displacement and visual receptors. These extension reflexes provide a method for the reliable initiation of postural abdominal extensions. They have been used to elicit abdominal extensions in studies concerned with motor neurone reciprocity during extension (Larimer & Eggleston, 1971), generic differences in the neuronal systems controlling extension (Page, 1975*b*) and load compensation (Sokolove, 1973).

However, because of the complex nature of the platform removal and crayfish uplift stimuli, the usefulness of these reflexes as tools for generating abdominal extensions in experiments which examine the neuronal control of postural abdominal movement is limited. As described in this report, postural abdominal extensions can be elicited by mechanical stimulation of single thoracic legs in the crayfish, *Procambarus clarkii*. Flexion of the mero-carpal joint in a single leg is a sufficient stimulus to elicit a vigorous extension response. The extensions are accompanied by stereotyped movements of most cephalic, thoracic and abdominal appendages. Since these abdominal extension responses can be evoked by simple mechanical stimulation of single leg joints, they provide attractive reflex preparations for the investigation of the neuronal systems which control abdominal movement.

METHODS

The experimental animal

Crayfish, *Procambarus clarkii*, obtained from Carolina Biological Supply Co., Burlington, N.C., were maintained in large aquaria, and fed dried dog food.

In most instances the 1st (the chelipeds), 3rd and 4th pairs of thoracic legs were autotomized. Leg removal reduced interactions between adjacent legs ('hand holding') which might interfere with mechanical stimulation of the legs. Following removal of the six legs the crayfish were kept for a recovery period of one week before their reflex responses were examined. These animals were healthy and vigorous; th

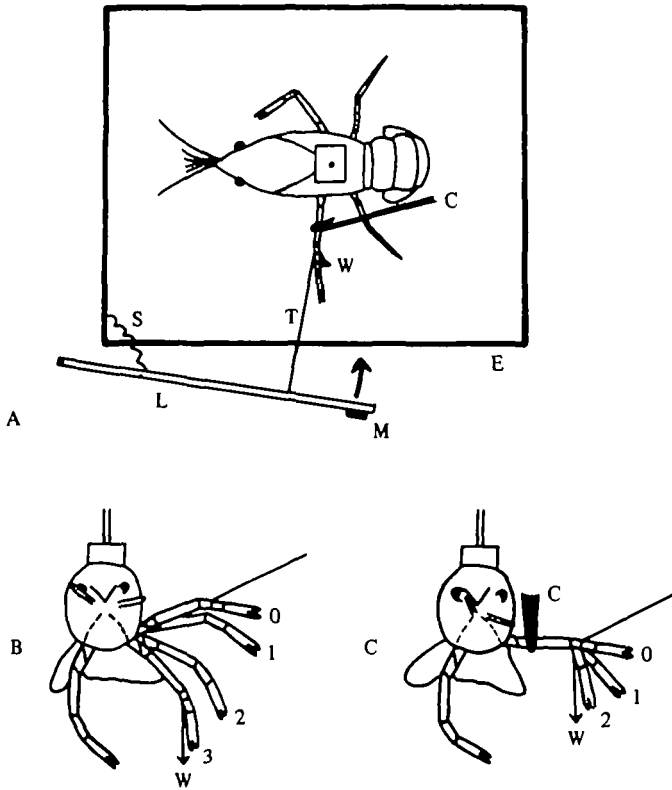


Fig. 1. Experimental preparation. (A) Dorsal view of crayfish in chamber before MCF stimulation. Arrow indicates lever movement which initiates MCF stimulus. C, Merus clamp; E, chamber wall; L, lever; M, electromagnet; S, spring; T, thread; W, 5 g weight. (B) WLD stimulation. Numbers indicate leg position in single frame (filmed at 24 frames/s). Stimulation occurred between frames 0 and 1. W is 5 g weight. (C) MCF stimulation. Identical to B except that C is merus clamp.

remaining 4 legs (the 2nd and 5th pairs) were sufficient for execution of a broad range of normal behaviour patterns including feeding, forward and backward walking, escape and swimming.

Several days before an animal was used in an experiment the antennae were shortened to a length of 2 cm and a plastic mount was attached to the posterior dorsal carapace of the thorax with Eastman 910 adhesive (Eastman Chemical Products).

Crayfish were suspended in the centre of an experimental chamber from a metal rod which was screwed into the plastic mount (Fig. 1 A). Care was taken to position the crayfish so that none of its appendages (antennae, legs, uropods, etc.) could contact a solid surface (i.e. the walls and floor of the chamber) since contact with a supporting surface often inhibited the abdominal extension response.

Bright acrylic paint was applied to the eyes, antennae, maxillipeds and alternate segments of each leg (coxa, ischium, carpus and dactyl), at least 24 h before an experiment, to heighten the contrast in super-8 films (see below). In some early experiments the eyes were occluded with a mixture of petroleum jelly and powdered charcoal.

- Leg joints were immobilized by encasing them in dental cement. The leg was held in an extended position, projecting laterally out from the side of the thorax, was dried carefully and one or more joints were covered with Caulk Grip Cement (L. D. Caulk Co., Milford, Delaware) which hardened within 20 min. Once the cement hardened the animal was returned to a water-filled chamber for a recovery period of 30 min before examination of its extension responses.

The water in the chamber was aerated continuously. Most observations were conducted at room temperature (20–24 °C). Films were obtained at somewhat higher temperatures (24–27 °C) as a result of the heat produced by the photoflood lamps.

Mechanical stimulation

Two mechanical stimulus situations were used to elicit abdominal extension: (1) whole-leg depression (WLD) (Fig. 1 B); (2) flexion of the mero-carpal joint (MCF) (Fig. 1 C). Both the WLD and the MCF stimuli were applied to a leg (usually one of the 2nd legs) which was held in a partially elevated horizontal position, extending laterally out from the thorax towards the chamber wall (Fig. 1). The leg was held in this position by a thread that was tied around the middle of the carpus (or propus in the joint immobilization experiments) and connected to a hinged lever which was positioned alongside the chamber (Fig. 1 A). The spring connected the lever to the chamber. The leg was pulled into an elevated, extended position by moving the lever away from the chamber and thereby stretching the spring. An electromagnet was used to hold the lever (with stretched spring) away from the chamber. The WLD stimulus was initiated by turning the electromagnet off to release the lever which was pulled towards the chamber by the spring. Movement of the lever towards the chamber caused the leg to drop from an outstretched horizontal position to a depressed vertical position (Fig. 1 B). This was the WLD stimulus. To ensure a constant WLD stimulus a 5 g weight was suspended from a string tied to the ventral aspect of the carpus. When the leg was released, the falling weight pulled the leg into a depressed vertical posture.

The stimulus conditions used to generate an MCF stimulus were identical to those which produced the WLD stimulus except that leg movement was confined to the distal leg joints. A clamp (Figs. 1 A, C) was attached to the middle of the merus to hold the proximal segments of the leg (coxa, basis, ischium, merus) in a stationary position - partially elevated, extending laterally out from the side of the thorax - during MCF stimulation. Since the lever was connected by a thread to the middle of the carpus, release of the lever (turning the electromagnet off) flexed the mero-carpal joint (Fig. 1 C). This was the MCF stimulus. Rapid flexion was ensured by suspending a 5 g weight from the middle of the carpus.

Measurement of reflex responses

Reflex responses were filmed at 24 frames/s with a Bolex 160 super-8 camera. The camera was positioned to record both frontal and lateral views of the responding animals (see Figs. 3 and 4). Frontal views were filmed for 39 WLD and 40 MCF responses of eight animals while lateral views were recorded for 36 WLD and MCF responses of the same eight animals. While the frontal views provided inform

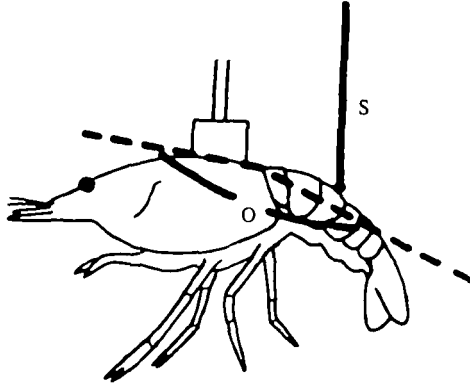


Fig. 2. Extension measurement. Abdominal position was determined by measuring the angle 'O' which was formed by the intersection of lines projecting from the posterior dorsal thorax and from the dorsal surface of the 5-6 abdominal joint. S, Mechanical stop used to measure extension force.

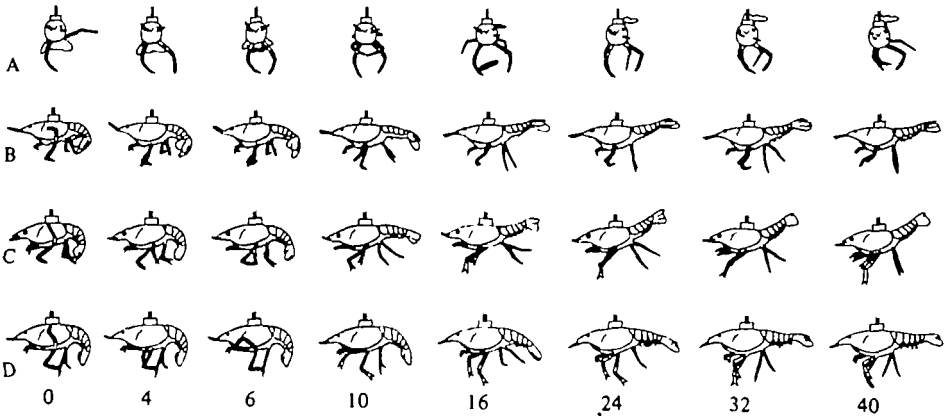


Fig. 3. WLD responses. Drawings of single frames from film made at 24 frames/s. Stimulation occurred between frames 0 and 1. Frame 24 is about 1 s after stimulus. A and B are successive responses from the same animal. B, C and D are different animals. Muscle recordings for B, C and D are in Fig. 5. Numbers indicate frame.

tion concerning the responses of the cephalic appendages to the WLD and MCF stimuli, detailed analysis of the films was confined to those which showed lateral views of the responses to WLD and MCF stimulation. In several instances, as a result of obscuration of an appendage or other characteristics of the filmed response, the total number of films which were analysed was less than the 36 WLD and 40 MCF trials which were filmed. For example, in several of the films movements of the swimmerets were obscured with the result that swimmeret responses were analysed in only 34 WLD and 36 MCF trials.

The onset of the WLD or MCF stimulus was recorded by attaching a light-emitting diode - which was connected in series with the electromagnet - to the side of the chamber. A single frame Bell and Howell no. 4712 movie projector was used to analyse the films. Single frames (usually frames 0, 1, 2, 3, 4, 6, 10, 16, 24, 32 and 40)

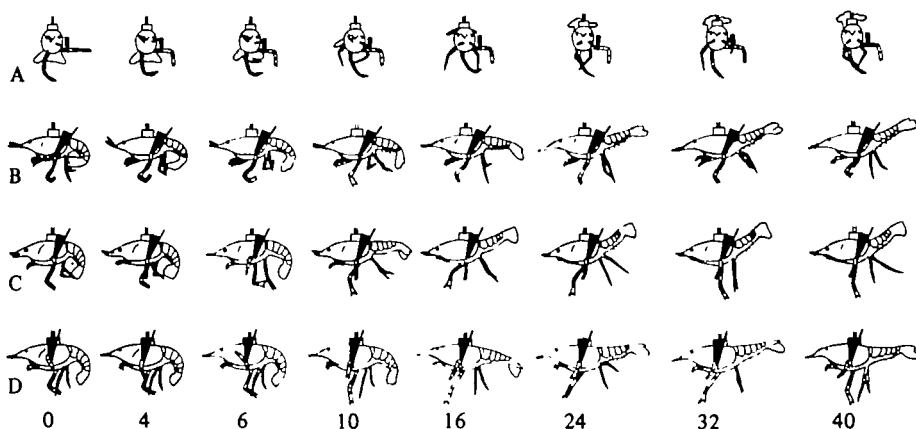


Fig. 4. MCF responses. A and B are successive responses from the same animal. B, C and D are the same animals as in B, C and D in Fig. 3. Muscle recordings for B, C and D are in Fig. 6. See Fig. 3 for other comments.

were projected on to tracing paper and drawn. Measurements of abdominal position were obtained by measuring the angle formed by two lines, one formed by the dorsal surface of the posterior thorax and the other extending from the dorsal aspect of the posterior edge of the thorax to the dorsal surface of the joint between the 5th and 6th segments (Fig. 2). Measurements of latency of movement were made in units of frames, which represents intervals of 41.6 ms, and were rounded to the nearest millisecond.

The force exerted by the extending abdomen was measured by positioning a mechanical stop to block extension of the third abdominal segment (Sokolove, 1973). The mechanical stop was a T-shaped brass bar which was attached to a FT03 Grass force transducer. The T-bar was placed in a standardized position in contact with the tergum of the flexed 3rd abdominal segment: directly over the middle of the segment, 2.5 mm ventral to a horizontal line extending caudally from the dorsal surface of the posterior thorax (Fig. 2). Force generated by abdominal extension against the T-bar was measured with the transducer and displayed on a storage oscilloscope. The maximum force (peak force) generated during the extension was determined by visual inspection of the trace stored on the oscilloscope screen.

Muscle potentials were recorded from the superficial extensor muscles of the 2nd abdominal segment by inserting a pair of 100 μm diameter insulated copper wires through the cuticle overlying the muscles. Responses were recorded for 79 WLD and 75 MCF responses in eight animals. The potentials were amplified with a Grass P15 preamplifier, displayed on a storage oscilloscope and stored with an Ampex SP700 FM tape-recorder. Permanent records were obtained by replaying the tapes into an oscilloscope and filming the potentials with a Grass kymograph camera.

RESULTS

Complex reflex responses, which included both postural extension of the abdomen and characteristic movements of the cephalic, thoracic and abdominal appendages were elicited by WLD (Fig. 3) or MCF (Fig. 4) stimulation of one or more thoracic walking legs pairs 2–5, inclusively. In contrast, neither WLD nor MCF stimulation evoked postural extension of the abdomen when applied to the chelipeds (1st pair of legs).

Stimulation of a single leg was sufficient to initiate a vigorous response. Abdominal extension responses could be evoked by WLD stimulation of the 2nd, 3rd, 4th or 5th legs. Since simultaneous WLD stimulation of the 2nd and 5th legs produced an extension response that was statistically no larger than the extension response which was generated when a single leg was stimulated (Table 1), the reflex responses which are described in the remainder of this report were elicited by stimulation of a single 2nd leg.

Localization of reflex sensitivity in the leg

The intensity of the abdominal extension elicited by WLD stimulation could be reduced by the immobilization of one or more of the leg joints with dental cement (see Methods). Immobilization of the 4 principal leg joints – the thoracic-coxal (T-C), coxo-basal (C-B), mero-carpal (M-C), and carpo-propal (C-P) – completely blocked the reflex response (Table 2).

The contribution of each leg joint to the abdominal extension response was examined in a series of experiments in which the leg stimulus (release of the thread attached to the propus – see Methods) was restricted to a single joint by the immobilization of all other leg joints with dental cement. These experiments demonstrated that stimulation of the T-C, B-C and M-C joints would initiate abdominal extension responses (Table 2). Flexion of the M-C joint elicited extensions which were 84 % as strong as those generated before immobilization of any leg joints. Much weaker extensions were initiated in response to stimulation of the C-B joint (38 % of intact leg control) or T-C joint (21 % of control). The sum of these two intensities is similar to the intensity of the response produced by simultaneous stimulation of the C-B and T-C joints (66 % of control). In contrast to stimulation of the more proximal leg joints, stimulation of the C-P joint failed to elicit an abdominal extension in eight of ten trials. The effects of separate stimulation of the baso-ischial (B-I) and ischio-meral (T-M) joints were not examined.

Appendage movement: WLD stimulation

The characteristic sequences of movements which the cephalic, thoracic and abdominal appendages generated in response to WLD stimulation of the left 2nd leg are shown in Figs. 3 and 5. Movements which were most commonly recorded in the first ten frames (about 450 ms) include: elevation and lateral rotation of the antennae; elevation and extension of the 3rd maxillipeds; elevation, extension and protraction (sometimes retraction) of right 2nd leg; elevation, extension and retraction both 5th legs; beating of the swimmerets (in 23 of 34 responses); and extension

Table 1. *Comparison of abdominal extensions evoked by WLD stimulation of the 2nd and/or 5th legs*

Leg	Extension force (g)	Range (g)	N of trials
2nd	22.8; S.E. = 2.9	0-53	25
5th	26.7; S.E. = 3.5	0-53	25
2nd and 5th	36.8; S.E. = 2.3	2-75	50

Extension force is peak force measured with force transducer blocking extension of 3rd abdominal segment. Values are means with standard errors. Student *t* test shows no significant differences between values (0.05 level of significance). Data obtained from measurements on 5 different animals.

Table 2. *Effects of leg depression stimulation of the joints of the 2nd leg*

Moveable joints	Immobilized joints	Abdominal extension Force (g)	% of Control value*	No. of trials	No. of animals
All	None	19.2; S.E. = 1.3	100 (19.2)	53	10
T-C	C-B, B-I, I-M, M-C, C-P	3.4; S.E. = 1.0	21 (17.3)	10	3
C-B	T-C, B-I, I-M, M-C, C-P	6.7; S.E. = 1.8	38 (17.3)	13	2
T-C, C-B	B-I, I-M, M-C, C-P	14.4; S.E. = 1.2	66 (21.8)	32	4
M-C	T-C, C-B, B-I, I-M, C-P	14.7; S.E. = 2.6	84 (16.4)	15	2
C-P	T-C, C-B, B-I, I-M, M-C	0.9; S.E. = 0.6	5 (17.3)	10	2
None	All	0	0	20	4

Extension force is expressed for each set of experiments as a percentage of the abdominal extension force (in g) generated by stimulation of the free leg (before joint immobilization).

* Control values differ because different animals were used. Joint abbreviations are in text. Measurement of abdominal extension force is the same as in Table 1.

Table 3. *Initial movements of contralateral leg in response to stimulation of the second leg*

Movement of contralateral 2nd leg	No. of WLD	No. of MCF	% of WLD	% of MCF
Protraction	16	19	44	47.5
No T-C movement	7	9	19	22.5
Retraction	13	12	36	30
Depression	11	24	30.5	60
No C-B movement	5	3	14	7.5
Elevation	20	13	55.5	32.5
Extension	27	31	75	77.5
No M-C movement	5	7	14	17
Flexion	4	2	11	5

Data obtained from a single frame analysis of first 10 frames (about 430 ms) of the filmed response. Abbreviations defined in text. WLD and MCF stimuli were applied to left 2nd leg to initiate movement responses. Data from 36 WLD and 40 MCF responses of 8 animals.

of the abdomen. As indicated by the data summarized in Table 3 there was considerable variability in right 2nd leg movements, especially movements of the T-C (protraction-retraction) and B-C (depression-elevation) joints. In about half of the responses small brief flexions of the maxillipeds, right 2nd leg and both 5th legs preceded these movements.

The latencies for the initiation of appendage movement ranged from 1 frame (about 42 ms) to 14 frames (about 583 ms) (Table 4). Movement of the cephalic and thoracic appendages preceded the initiation of abdominal movement. The initiation of rig

2nd leg movement had a significantly greater latency than the other cephalic and thoracic appendages.

The above initial responses were followed by a series of movements (Fig. 3, frames 11–40; about 450–1700 ms) which include: depression and forward protraction of the antennae; depression of the 3rd maxillipeds; increased extension, elevation and protraction of the right 2nd leg (this was variable—sometimes the right 2nd leg was flexed, depressed and retracted); elevation, retraction and flexion followed by depression, extension and protraction of both of the 5th legs. In some instances movements of the two 5th legs alternated. For example, the right 5th leg would depress, extend and protract while the left 5th leg was elevated, retracted and flexed. These movements were usually followed by elevation, flexion and retraction of the right 5th leg, and depression, extension and protraction of the left 5th leg. Both swimmeret beating (in 23 of 34 responses) and abdominal extension continued for the duration of the response.

Swimmeret beating was observed in only 23 of the 34 filmed responses to WLD stimulation. In the first 40 frames (about 1.7 s), the mean number of beats was 4.3 (S.E. = 0.4) with a range of 1–7 beats.

Detailed records of uropod movement were not available from the films which only recorded frontal and lateral views of the reflex response. However, visual observations revealed that the uropods were both extended and abducted during the course of the reflex response.

In contrast to the other appendages the eyes rarely moved following WLD stimulation. In only 2 of the 75 WLD responses filmed, the eyes moved with a flicking movement—upward movement followed by downward movement—in the 2nd and 3rd frames following WLD stimulation.

Appendage movement: MCF stimulation

The movements of the cephalic, thoracic and abdominal appendages in response to MCF stimulation (Fig. 4) were similar to those described above for WLD stimulation. In the first 450 ms of the MCF response, movements of the antennae, eyes, 3rd maxillipeds and right and left 5th legs were identical to the movements evoked by WLD stimulation (Fig. 4). Only movements of the right 2nd leg—extension, depression and protraction—differed significantly from those evoked by the WLD stimulus (Table 3). The right 2nd leg tended to elevate in response to WLD stimulation while it depressed when stimulated by MCF. The coupling of swimmeret beating to the MCF response was less than that observed in WLD responses; swimmeret beating was observed in 17 of the 36 responses to MCF stimulation.

Latencies for initiation of appendage movement in response to MCF stimulation were similar to those measured for WLD responses (Table 4) except for the right 2nd leg. Movement of the right 2nd leg was initiated after 3.0 (S.E. = 0.2) frames (about 125 ms) which is significantly shorter than the 4.9 (S.E. = 0.5) frames (about 204 ms) latency for responses to WLD stimulation.

The appendage movements observed in the later stages of the MCF response (about 450–1700 ms) differed somewhat from those observed in response to WLD stimulation (Fig. 4). The most common appendage movements were elevation of the

Table 4. *Latencies for initial movement of appendages in response to stimulation of the second leg*

Body part	Latency (frames)	Range (frames)	Latency (ms)	No. of trials
WLD stimulation				
Antennae	3·6; S.E. = 0·5	2-3	159	36
3rd maxillipeds	3·3; S.E. = 0·3	1-9	138	36
Right 2nd leg	4·9; S.E. = 0·5	1-14	204	36
Right 5th leg	3·5; S.E. = 0·3	1-11	146	36
Left 5th leg	4·0; S.E. = 0·4	1-8	167	36
Abdomen	5·3; S.E. = 0·5	3-10	221	36
MCF stimulation				
Antennae	3·3; S.E. = 0·4	1-12	138	37
3rd maxillipeds	3·8; S.E. = 0·2	1-11	158	39
Right 2nd leg	3·0; S.E. = 0·2	1-7	125	37
Right 5th leg	3·9; S.E. = 0·4	2-12	163	35
Left 5th leg	4·0; S.E. = 0·4	1-12	167	38
Abdomen	5·4; S.E. = 0·4	3-11	225	39

Measured from single frames of films obtained at 24 frames/s. The Student *t* test showed that the following latencies were significantly different (0·01 level of a significance): WLD abdomen *v.* other WLD body parts; WLD right 2nd leg *v.* other WLD appendages; MCF abdomen *v.* other MCF body parts; WLD right 2nd leg *v.* MCF right 2nd leg. Data were obtained from films of WLD and MCF responses of 8 animals. Values of *N* less than 40 for the MCF responses result from the obscuration of the initial movements in some of the films.

right second leg (sometimes with weak extension): retraction and elevation followed by depression, protraction and extension of both 5th legs. Often movements of the two 5th legs alternated as described for WLD responses. Movements of the other appendages – antennae, eyes (flicked once in 80 responses), swimmerets and uropods – were identical to those observed during WLD responses. Descriptions of uropod movement are based upon visual observations only.

Abdominal extension movement: WLD v. MCF stimulation

Abdominal extension movement was initiated by WLD stimulation with a latency of 5·3 (S.E. = 0·5) frames (about 221 ms) (Table 4). Before WLD stimulation the mean abdominal position was 151·9° (S.E. = 1·8) (Table 5). Maximal abdominal extension was attained within the first 24 frames (1 s) for 29 of the 36 responses which were filmed. The mean abdominal position recorded in the 24th frame was 194·8° (S.E. = 2·6) (Table 5). Therefore the average response to WLD stimulation was an extension of 42·9° (S.E. = 2·2). The velocity of this average extension – measured between frame 6 (about 250 ms) and frame 16 (about 667 ms) – was 63·1° (S.E. = 2·4) per s.

The latency for initiation of abdominal extension movement in response to MCF stimulation (Table 4) (5·4; S.E. = 0·4 frames or about 225 ms) was identical with the latency measured from the films of WLD responses. Likewise each of the other measurements of the abdominal extension response obtained from the films of MCF responses was identical with the values listed in the preceding paragraph for responses to WLD stimulation. These include: the initial position of the abdomen (154·0°; S.E. = 2·2); the position of the abdomen at 24 frames (1 s) (191·3°; S.E. = 2·4) and the velocity of extension between frames 6 and 16 (62·8° per s, S.E. = 2·6).

Table 5. *Abdominal position during extension in response to stimulation of the second leg*

Response ...	WLD		MCF	
	Mean abdominal position (deg)	Range (deg)	Mean abdominal position (deg)	Range (deg)
0	151.9; S.E. = 1.8	132-181	154.0; S.E. = 2.2	132-178
4	154.7; S.E. = 1.9	132-181	156.7; S.E. = 2.4	132-189
6	161.4; S.E. = 2.1	132-181	159.5; S.E. = 3.8	134-197
10	175.0; S.E. = 2.7	136-191	172.4; S.E. = 2.5	144-205
16	187.7; S.E. = 3.0	147-208	185.7; S.E. = 2.7	145-216
24	194.8; S.E. = 2.6	158-214	191.3; S.E. = 2.4	158-216

Abdominal position was measured (in degrees) from the angle formed between the dorsal surfaces of the thorax and abdomen (see Methods and Fig. 2). *N* = 36 for WLD responses and 40 for MCF extensions. Data obtained from single frame analysis of films produced at 24 frames/s for 8 animals.

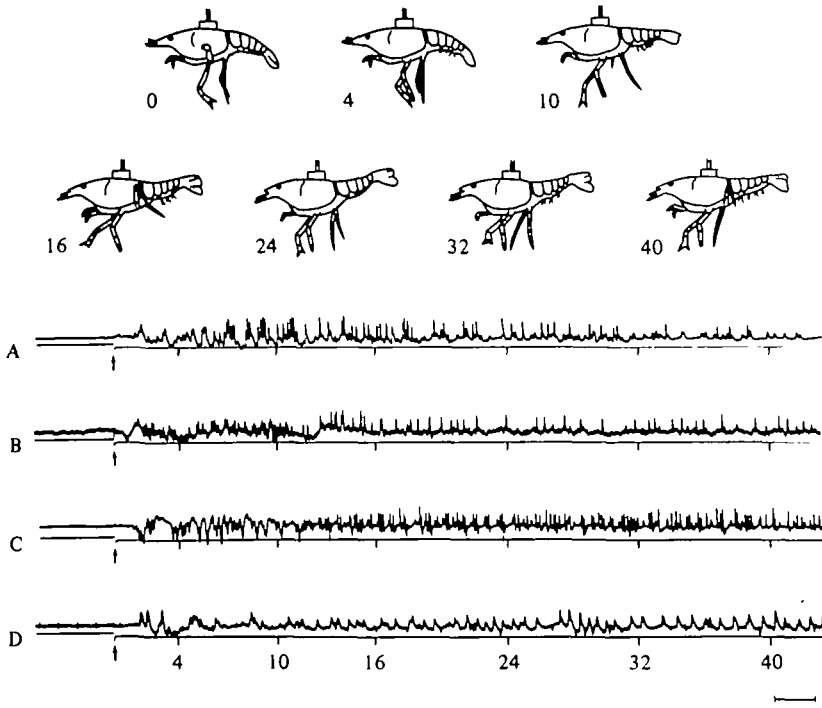


Fig. 5. Extensor muscle activity during WLD responses. Single-frame drawings for A are at the top of figure. Single-frame drawings for muscle potential records B, C and D are found in Fig. 3B, C and D respectively. Arrow indicates WLD stimulus. Numbers indicate approximate time of single frames shown in drawings. Calibration 100 ms.

Superficial extensor activity: WLD v. MCF stimulation

WLD stimulation always elicited a train of potentials in the superficial extensor muscles of the 2nd abdominal segment (Fig. 5) with a latency of 53.7 (S.E. = 2.1) ms (range 26-148 ms, measured from 79 responses of 8 crayfish). Both the rate and amplitude of the muscle potentials increased during the first 500-1000 ms (Fig. 5). Muscle potential activity gradually decreased as the abdomen attained a position of maximum extension.

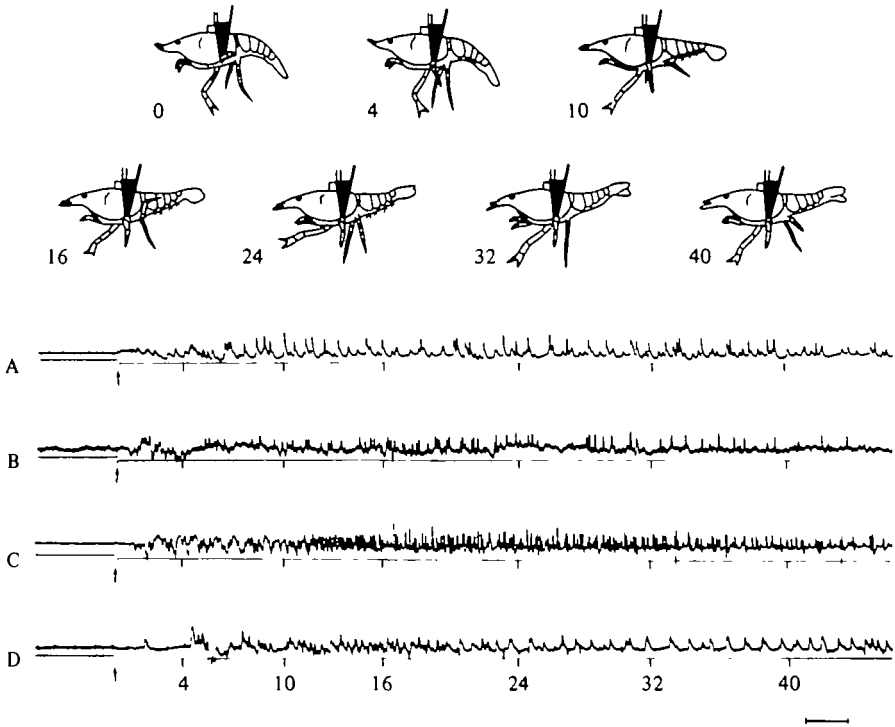


Fig. 6. Extensor muscle activity during MCF responses. As Fig. 5 except that single frame drawings for muscle potential records B, C and D are shown in Fig. 4 B, C and D respectively.

All muscle potentials recorded during extensions elicited by WLD stimulation were small-amplitude, tonic potentials characteristic of the tonic superficial extensor musculature. In 5 out of the 79 WLD responses the crayfish responded to the WLD stimulus with an initial (1st 500 ms) series of rapid tail flips before generating a tonic abdominal extension. The large phasic muscle potentials which were recorded during these tail flips resulted from activity of the deep phasic abdominal musculature.

The recordings which were obtained from the superficial extensor muscles in response to MCF stimulation (Fig. 6) were similar to those elicited by WLD stimulation. The mean latency for the initiation of muscle potential discharge in response to MCF stimulation was 50.0 (S.E. = 2.0) ms (75 responses from 8 crayfish). In contrast to recordings of WLD responses tail flip responses were never observed in response to MCF stimulation (75 responses, 8 animals).

Termination of WLD and MCF responses

While detailed analyses of WLD and MCF responses were confined to the first 40 frames (1.66 s) of the film record, most crayfish maintained their abdomens in an extended posture for a period of 10 or more seconds. Throughout this period there was minimal movement of the appendages. Usually they maintained the positions and postures that they adopted during the first 2 s of the response. In some animals, slow alternate depression and elevation movements of the right and left 5th leg

Continued. Animals which responded to WLD or MCF stimulation with an initiation of swimmeret beating continued to beat their swimmerets for the duration of the response.

Eventually, after a period of 10–20 s, the crayfish returned its abdomen to its initial partially flexed posture. Return of the abdomen to a flexed position was accompanied by movement of the appendages.

DISCUSSION

Reflex responses which include both postural extension of the abdomen and movements of the cephalic, thoracic and abdominal appendages can be evoked in the crayfish *Procambarus clarkii* by the mechanical stimulation of one or more legs. Movement of a single leg joint is sufficient to initiate a strong reflex response (Table 2; Figs. 4 and 6). The stimuli in order of decreasing effectiveness are (Table 2): (1) depression of the whole leg (WLD – Fig. 1B); (2) flexion of the M–C joint (MCF – Fig. 1C); (3) depression of the T–C and C–B joints; (4) depression of the C–B joint; and (5) depression of the T–C joint. The sensitivity of the reflex to small amplitude depression movements of the T–C is surprising since movement of the T–C joint occurs in the frontal plane (protraction-retraction) (Ayers & Davis, 1977). The strength of the abdominal extension response which is produced by the leg depression stimulus may reflect the fact that the loosely articulated T–C joint is both depressed and slightly protracted by the stimulus.

Postural abdominal responses can also be elicited by removal of a platform supporting the legs of the crayfish *Procambarus clarkii* (Larimer & Eggleston, 1971). This platform reflex can be initiated by stimulation of a single leg (C. Page – unpublished results). Immobilization of the T–C and C–B joints with dental cement produces a 70% reduction in the strength of the extension. Neither immobilization of the remaining leg joints (BI, IM, MC, CP and PD), nor destruction of the statocysts, nor removal of all legs by autotomization eliminates the residual (30%) response. Since the residual response can be eliminated by coating the thorax of the legless animal with petroleum jelly, it is probably mediated by vibration sensitive receptors which respond to water currents generated when the platform is removed (C. Page – unpublished results).

The defence reflex of *P. clarkii*, which includes raising and opening the chelipeds, elevation of the thorax and abdominal extension, is a stereotyped response to visual (Glantz, 1974; 1978) and tactile (Tsukada, 1974) stimulation. Excitation of motion detector units in the optic nerve will elicit the response (Glantz, 1974, 1978). In the spiny lobster, *Palinurus vulgaris*, tilting of the substrate initiates a series of responses which involve compensatory movements of the antennae, eyes, legs, abdomen and uropods (Schone *et al.* 1976). These responses can be elicited by tilt stimulation of a single leg. Immobilization of the C–B joint eliminates the responses to substrate tilt.

Movement sensitive leg receptors in the crayfish include both chordotonal organs at the T–C, C–B and M–C joints and the muscle receptor organ at the T–C joint and the myochordotonal organ at the M–C joint (Alexandrowicz & Whitear, 1957; Clarac & Masson, 1969; Wales *et al.* 1970; Bush, 1976). Other receptor systems which might be affected by WLD and/or MCF stimulation include the cuticular stress

receptors at the B-I joint (Wales, Clarac & Laverack 1971; Clarac, 1976; Mill, 1970) and cuticular hairs at the M-C joint (Laverack, 1976). The strength of the extension which is evoked by MC flexion suggests that excitation of flexion sensitive receptors in the chordotonal (MC_1 and MC_2) and myochordotonal organs at the MC joint is sufficient to initiate the extension reflex.

Crayfish whose legs contacted the walls of the chamber were often unresponsive to WLD and MCF stimulation. Contact between legs ('hand-holding') usually reduced or blocked the extension response. The loss of contact between the distal leg segments (propus and dactyl) and a supporting surface has been shown to be associated with the initiation of abdominal extension in the platform reflex (Larimer & Eggleston, 1971) and the uplift reflex (Page, 1975*b*).

Movements of the cephalic, thoracic and abdominal appendages were coupled to varying degrees with the abdominal extension responses evoked by WLD or MCF stimulation. The initial movements of the antennae, maxillipeds, 5th legs and uropods were very stereotyped; they generated in response to each bout of stimulation. The absence of eye movement was also a consistent component of both WLD and MCF responses. The responses of the contralateral 2nd leg and the swimmerets were more variable.

Except for the 79 ms difference in the latency for the initiation of contralateral 2nd leg movement (Table 4), the reflex responses which were evoked by WLD and MCF stimulation were very similar. The two stimulus conditions differ both in the joints affected and the degree of restraint which they impose upon the leg. During MCF stimulation, the proximal leg was held in a stationary elevated position while the M-C joint was flexed, while WLD stimulation released the leg from an elevated position, thereby depressing the C-B joint and removing all restraint on leg movement.

Since different receptor systems are presumed to be affected by MCF and WLD stimulation, the similarity of the reflex evoked by MCF and WLD stimuli is somewhat surprising. This similarity can be explained if both responses are mediated by common sets of interneurons which receive excitatory inputs from more than one type of leg receptor, including those sensitive to depression of the C-B joint and those responding to M-C joint flexion. A large number of interneurons which respond to leg movement stimulation have been described in circumoesophageal connectives (Wiersma, 1958) and the connectives between the thoracic and abdominal nerve cords (Wiersma and Bush, 1963).

To initiate movement of cephalic, thoracic and abdominal appendages, the sensory excitation resulting from WLD or MCF stimulation must activate both ascending and descending interneuronal pathways in the nerve cord. The complexity of these pathways and the number of interneurons which compose them is unknown, although the short latency which was measured (total latency for mechanical stimulation, sensory response and neuronal conduction was about 50 ms) for the appearance of potentials in the superficial extensor musculature suggests that a relatively direct pathway activates the abdominal musculature.

The nerve cord connectives contain many premotor interneurons (command fibres) which when stimulated with trains of electrical pulses elicit coordinated movements of the appendages and abdomen (Atwood & Wiersma, 1967; Bowerman

& Larimer, 1974*a*; *b*). Several of these premotor interneurons elicit coordinated postural extensions of the abdomen when stimulated electrically (Atwood & Wiersma, 1967; Evoy & Kennedy, 1967; Fields *et al.* 1967; Bowerman & Larimer, 1974*a*; Page, 1975*a*). The activation of one or more of these 'command fibre' interneurons by the afferent activity generated in response to WLD or MCF stimulation would provide a rapid conducting pathway from the thoracic nerve cord to the abdominal motor centres. Likewise one or more command fibres could provide the pathway for transmission of activity evoked by WLD or MCF stimulation to the higher neural centres (supra-oesophageal and sub-oesophageal ganglia) to initiate antennal and maxilliped movements.

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