

CRAYFISH EXTRARETINAL PHOTORECEPTION

I. BEHAVIOURAL AND MOTONEURONAL RESPONSES TO ABDOMINAL ILLUMINATION

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SUMMARY

1. Stimulation of blinded and sighted crayfish with ventrally directed light evokes a slow tail flexion response or a tail flexion accompanied by backward walking. The response latencies and durations of sighted animals are shorter than those of blinded animals, which indicates that visual inputs can speed a response which can be released by extraretinal photoreceptors alone.

2. Recordings from electrodes implanted in intact, freely behaving animals demonstrate that ventral illumination tonically excites abdominal postural flexor motoneurons. The motoneurone discharge occurs first in caudal segments and then spreads rostrally, as does abdominal flexion around each segmental joint.

3. Illumination of individual abdominal ganglia (A2–A5) tonically excites a similar flexor motoneurone response in cells of the stimulated ganglion and more caudal ganglia. Swimmeret motoneurons are also tonically excited by this stimulus. These responses can be evoked in isolated abdominal nerve cords, indicating that extraretinal photoreceptors present in these ganglia activate motor circuits that are local to the abdomen.

4. Stimulation of A6 excites the caudal photoreceptor neurones, but only excites flexor motoneurons if the abdominal ventral nerve cord is connected to the rostral part of the CNS. The motoneurons respond with repeated bursts of activity that long outlast the stimulus or the initial high-frequency burst of the caudal photoreceptor neurones. These motoneurone responses are similar to those evoked by stimulation of command fibres that also evoke backward walking (Kovac, 1974a).

INTRODUCTION

For fifty years it has been known that crayfish respond to light directed at the abdomen with increased locomotor activity (Prosser, 1934; Welsh, 1934). This motor activity has been thought to be due to the photoexcitation of the two abdominal caudal photoreceptor (CPR) neurones (Prosser, 1934; Kennedy, 1958, 1963; Wilkens & Larimer, 1972, 1976). These cells respond to the sudden onset of bright white light

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directed at the sixth abdominal ganglion (A6) with a high-frequency burst, followed by a long lasting, lower-frequency train of impulses. The nature of the motoneuronal output released by photoexcitation of the CPR neurones has remained unclear, however, as has the role of other, unidentified photosensitive neurones discovered in the abdominal ventral nerve cord (Wilkins & Larimer, 1972).

This paper describes two distinct types of abdominal flexion behaviour, and their associated flexor motoneurone responses, that can be released by illumination of the crayfish abdomen. One of these types of behaviour is a simple postural flexion of the abdomen that can occur alone or accompanied by backward walking. Motoneurone activity which accompanies this type of behaviour can be evoked by photostimulation of the rostral five abdominal ganglia (A1–A5). The second behaviour pattern is a repetitive, alternating flexion and extension of the abdomen that is associated with simultaneous backward walking. Motoneurone activity that correlates with this behaviour pattern is released by photostimulation of the CPR neurones in A6 (Edwards, 1977).

METHODS

I performed experiments on two species of crayfish, *Procambarus clarkii* and *Procambarus acutus acutus*. Animals of both sexes 10–14 cm long were used. *P. clarkii* were obtained from Monterey Bay Hydroculture Farms, Monterey, Ca., while *P. acutus acutus* were obtained from a pond in Falmouth Mass. All experiments were performed on animals of both species. I observed no differences in the responses of the two species.

Behavioural experiments were done with intact animals, blinded animals, and animals with electrodes implanted around motor roots of abdominal ganglia. I placed single crayfish in an aquarium that had a translucent bottom and sides and dark adapted them for at least 1 h. When the animal was resting motionless with abdomen extended, I illuminated it from below with either white light from a 100 W incandescent source or long wavelength radiation from an incandescent heat lamp. Interstimulus intervals of 15 min in the dark allowed the experimental animal to become dark-adapted before each trial. The water in the aquarium was 5 cm deep and kept at 22 °C. The temperature at the bottom of the aquarium directly above the lamp was measured with a Keithley digital thermometer and thermocouple probe. Following onset of the white light, the water temperature on the bottom surface of the aquarium increased at a nearly constant rate of 0.18 °C s⁻¹. The responses of the animals were filmed with a 16 mm Beaulieu camera operating at 10 frames per second.

I blinded animals 24 h before experimentation by covering the entire surface of both eyestalks with opaque black paint. After completion of the experiments, I determined the effectiveness of the paint in maintaining blindness by inserting the luminous end of a fibre optic into the proximal cut end of each eyestalk. No light emerged from the eyestalks of those animals that had been effectively blinded. Data from animals whose eyestalks were not completely opaque were rejected.

Student's *t*-test was used in the analysis of behavioural responses to calculate levels of significance in tests for differences between sample populations.

To record abdominal postural motoneurone activity during the behavioural responses

For photostimulation, I implanted single 50 μm diameter silver hook electrodes around individual ganglionic roots in the abdomen. The intact, hooked nerve and electrode was drawn up into a 500 μm (diameter) by 1 mm (length) piece of polyethylene tubing that contained petroleum jelly, which served as an insulator. A reference electrode was fastened to the outside of the tubing, and the diame insulated leads of both electrodes were braided together. This bipolar lead was fastened with Eastman 910 adhesive to hard cuticle on the abdomen and thorax, and connected from there to an a.c. coupled amplifier. The length of tubing containing the nerve remained in the abdomen, covered over by a flap of soft exoskeleton and petroleum jelly. Good records could be obtained for at least 24 h while the animal was free to move within an aquarium.

I obtained intracellular recordings from isolated abdomen and isolated abdominal ventral nerve cord preparations. Each crayfish was first chilled for 15 min in an icebath, the abdomen was removed and the digestive tract irrigated with cold, oxygenated saline (Van Harreveld, 1936). The abdomen was pinned out in an extended position ventral side up in saline to expose the abdominal nerve cord. The ventral artery was removed and one or more ganglia were desheathed. The deep third ganglionic roots were cut to avoid exciting the fast flexor muscle. In isolated abdomen preparations, the ventral nerve cord was stabilized by pinning it out on a strip of Sylgard (Dow-Corning) inserted between the cord and the abdominal flexor muscle. In isolated cord preparations, the cord was removed from the abdomen and pinned out in a Petri dish lined with Sylgard and filled with saline. In both instances, ganglionic cell somata could be seen with reflected light after removing the ganglionic sheath from the ventral surface.

I stimulated individual ganglia with light from the luminous tip of a small (2 mm diameter by 30 cm length) fibre optic applied close to the ventral ganglionic surface. Stray light on other ganglia or tissue was minimal. Incident white light was provided by a 30 W tungsten incandescent lamp; monochromatic light at 546 nm was obtained by passing the white light through an interference filter. White and monochromatic light was focused onto the proximal end of the fibre optic after having passed through two heat filters.

RESULTS

Behavioural responses of sighted and blinded animals to ventral illumination

To study behavioural responses that might be released by abdominal photoreceptor excitation, I stimulated freely behaving crayfish with 15 s of bright ventral illumination after dark adapting them for an hour or more. (The abdominal ventral nerve cord is exposed to ventral illumination beneath the translucent ventral abdominal exoskeleton.) Experiments were performed on 25 sighted and 25 blinded (see Methods) animals; eight of the animals were tested in both conditions. Several trials were run on each animal; the 25 sighted animals were tested an average of nine times each (± 4 , s.d.), while the blinded animals were tested seven times each (± 3).

I observed several behavioural responses, in sighted and blinded animals, to ventral light stimulation. These included: slow or rapid tail flexion; backward or forward walking, either of which may accompany tail flexion; leg motion in place, which could also accompany tail flexion (Table 1).

Table 1. *Average frequencies of behavioural responses of sighted and blinded animals to ventral illumination*

Condition	Trials per animal*	No response	Flex	BW	FW	LM only
Sighted (<i>N</i> = 25)	9 ± 4	9 ± 11	70 ± 25	47 ± 29	16 ± 19	15 ± 18
Blinded (<i>N</i> = 25)	7 ± 3	16 ± 19	49 ± 30	33 ± 29	18 ± 22	16 ± 18

The frequency of each behavioural response was calculated for each animal, and those frequencies were averaged for sighted and blinded animals to obtain the values, expressed as percent total trials (\pm s.d.), presented in Table 1.

* Expressed as number, not percentage.

BW, backward walking; FW, forward walking; LM only, leg movement only.

For both sighted and blinded crayfish, the most frequent responses to ventral illumination were abdominal flexion and backward walking. Sighted animals gave both responses about 50% more often than blinded animals. For flexion, this difference is significant at the 1% level, while for backward walking the difference is significant only at the 10% level. These differences are in contrast to the similar response frequencies of the other behaviour patterns: forward walking, leg motion alone and no response all occur in sighted and blinded animals less than 20% of the time.

Tail flexion and backward walking frequently occurred together as parts of the same response. In sighted animals, 64% ($\pm 33\%$ s.d., *N* = 25) of flexion responses were accompanied by backward walking, while 90% ($\pm 18\%$, *N* = 22) of backward walking responses were accompanied by abdominal flexion. These frequencies were slightly lower in blinded animals: 50% ($\pm 38\%$, *N* = 19) of flexion responses were accompanied by backward walking, while 87% ($\pm 20\%$, *N* = 14) of backward walking responses were accompanied by flexion. When abdominal flexion is accompanied by backward walking, the flexion behaviour often consists of an initial complete flexion which is followed by a series of brief, incomplete extensions and flexions that persist during the backward walking. This behaviour pattern is quite similar to that evoked by threatening visual stimuli presented to crayfish moving freely on land (Kovac, 1974a).

When backward walking did not occur, the animal usually remained stationary during tail flexion. 33% ($\pm 33\%$, *N* = 25) of sighted animals remained stationary during flexion; this value, together with the backward walking frequency, account for 97% of flexion responses. The remaining 3% of flexion responses were accompanied by forward walking, turning movements or rolls to the side. Blinded animals remained stationary during flexion 36% ($\pm 37\%$, *N* = 19) of the time, which, when taken together with backward walking responses, indicates that 14% of flexion responses were accompanied by other behavioural activities. The flexion response of animals that remained stationary consisted of a single complete abdominal flexion without subsequent cycles of re-extension and flexion (Figs 1, 2). The single complete flexion began with an initial extension of rostral abdominal segments that raised the abdomen to allow the tailfan and caudal segments to flex and pass underneath rostral abdominal

Table 2. Mean latencies and duration, in seconds, of the responses of sighted and blinded animals to ventral illumination

Condition	Initial response latency	Flexion latency	Flexion duration
Sighted	2.7 ± 1.7 (19)	5.2 ± 2.7 (19)	1.7 ± 0.8 (19)
Blinded	4.5 ± 2.5 (21)	9.3 ± 4.1 (16)	3.8 ± 2.6 (16)
Significance level	0.01	0.001	0.01

Average response times were calculated for each animal from the measured times of between 3 and 13 trials; times in the table are the means (± s.d.) of those average response times.

The number of animals tested is given in parentheses with each time.

The third row indicates the level of statistical significance at which the sighted and blinded times are different.

segments. Thereafter, more rostral segments flexed in turn until the whole abdomen was tightly flexed. As each segment flexed, its swimmerets were thrust forward parallel to its longitudinal axis.

Cinematographic analysis made it possible to measure response latencies and durations for both sighted and blinded animals to ventral illumination (Table 2). Following stimulation, sighted animals responded with some movement (usually with leg motion) in slightly more than half the time of blinded animals. Similarly, the latency to the beginning of flexion and the duration of flexion were both about half as long for sighted animals as for blinded animals.

Although the change in temperature produced by the incandescent lamp at the bottom of the aquarium was small (approximately 2.7°C, see Methods), it was possible that the behavioural responses were primarily elicited by heat. This possibility was eliminated by control experiments in which four freely behaving animals were alternately stimulated with white light, from the incandescent lamp, and with red light and infra-red radiation, from a heat lamp. The animals were tested, first when sighted and then when blinded. The heat lamp produced nearly the same temperature change at the bottom of the aquarium (0.16°C s⁻¹) as the white light source. Averaged results for all animals are presented in Table 3. When stimulated with the heat lamp, both sighted and blinded crayfish failed to respond 60% of the time, whereas the same animals always responded to the white light stimulus when sighted, and failed to respond in only 5% of trials when blinded. Similarly, no sighted animals flexed in response to heat, while they flexed nearly 90% of the time in response to white light. Only one blinded animal flexed in response to heat (on two of eight trials), while white

Table 3. Responses of sighted and blinded animals to heat and white light

Condition	Trials per animal					
	No response	Flex	BW	FW	LM only	
Sighted, white light	5 ± 2	0	86 ± 18	73 ± 19	17 ± 14	3 ± 7
Sighted, heat	3 ± 1	58 ± 44	0	25 ± 29	17 ± 24	0
Blind, white light	4 ± 1	5 ± 10	77 ± 29	58 ± 20	17 ± 19	0
Blind, heat	4 ± 3	61 ± 13	6 ± 12	30 ± 21	3 ± 7	6 ± 13

Responses are averages of average responses of four animals, and are expressed as a percentage of the number of trials (± s.d.).

BW, backward walking; FW, forward walking; LM only, leg movement only.

light evoked flexions nearly 80 % of the time. These results indicate that white light is a much more effective stimulus in evoking the responses than heat. Those responses that did occur in response to the heat lamp are likely to have been evoked by the visible red light, rather than infra-red radiation. This conclusion is consistent with the lack of sensitivity of the caudal photoreceptors to heat in the temperature range (between 20 and 25 °C) in which these experiments were conducted (Larimer, 1967).

The responses of blinded animals are presumably due to the excitation of extraretinal photoreceptors, while the responses of sighted animals are presumably due to both visual and extraretinal excitation. These results indicate that extraretinal photoreceptors can release abdominal flexion, swimmeret protraction, backward walking and the other locomotor behaviour patterns described above. When visual stimulation is coupled with stimulation of extraretinal photoreceptors, the probability of flexion and backward walking responses are enhanced, while their latencies are reduced.

Responses of tonic flexor motoneurons to ventral illumination in blinded, freely behaving crayfish

To begin to study the neuronal responses that mediate the behaviour pattern described above, I implanted electrodes around 2nd and 3rd abdominal ganglionic roots in six blinded, freely behaving animals. The 2nd root (rt2) contains a mixed population of sensory neurones and tonic and phasic extensor motoneurons (Fields, 1966), while the superficial branch of the 3rd root (rt3s) contains abdominal tonic flexor motoneurons (Kennedy & Takeda, 1965; Evoy, Kennedy & Wilson, 1967; Wine, Mittenthal & Kennedy, 1974). The responses of the tonic flexor (TF) motoneurons in the rt3s of three abdominal segments (A2, A3 and A4) are given in Fig. 1 along with selected frames from the film of the animal's response. In that experiment, the animal was stationary with abdomen extended during the 1-h period of dark adaptation before the ventral light was turned on (see picture at 0.45 s after light on, Fig. 1). There was very little activity in the TFs of A2, A3 or A4 until 2.5 s after the light was turned on (Fig. 1). The abdomen remained stationary until nearly 5 s later when the rostral segments extended to allow the tailfan to flex and pass beneath more rostral segments (picture at 5.4 s, Fig. 1). The TFs in A4 reached their peak activity at about 5.2 s, while those in A3 peaked at 5.6 s and those in A2 peaked at 6.5 s. During this time, the abdomen flexed in a caudal to rostral direction and the swimmerets of each segment protracted as that segment flexed. The entire flexion was over at 8.8 s (Fig. 1), even though the TF motoneurons continued to fire rapidly for many more seconds.

The response of abdominal tonic extensor (TE) and TF motoneurons to ventral illumination of another blinded animal are presented in Fig. 2. In this animal, as in all others, the motoneurons displayed little or no activity while the animal rested in the dark with its abdomen extended (Fig. 2). Some activity began in the left root 2 of A3 (LA3rt2) almost immediately after the ventral light was turned on, but it did not become regular until 4 s later. The TFs in A3 delayed firing until 9 s after light on, when two units, presumably nos 3 and 4 (Kennedy & Takeda, 1965) began to fire. The TFs in A4 fired earlier, about 5 s after light on. Activity in root 2 peaked at 9.0 s, when the animal extended the rostral portion of the abdomen to permit the tail to pass

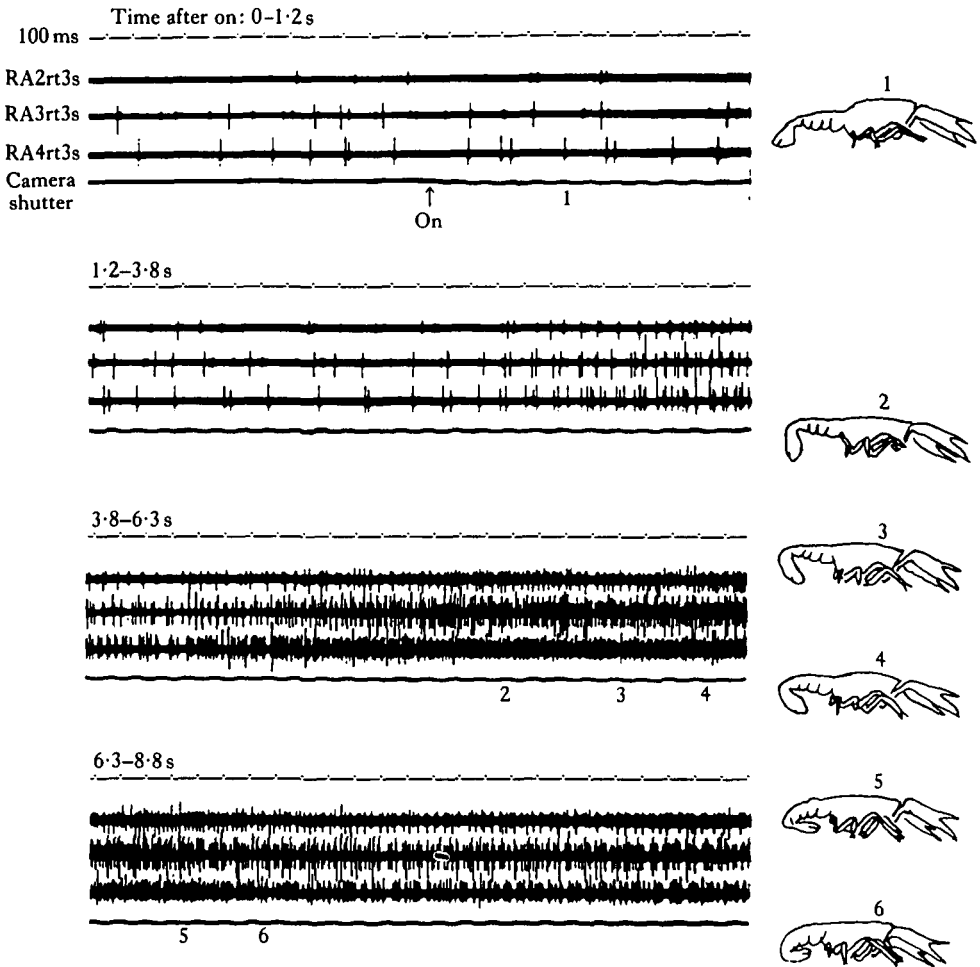


Fig. 1. Responses of a freely behaving blinded crayfish and its tonic flexor motoneurons to ventral illumination. Behaviour was filmed at 10 frames s^{-1} ; tracings of individual frames are shown on right. Concurrent tonic flexor activity was recorded from three segments, A2, A3 and A4, with implanted electrodes around right segmental superficial third roots. Numbers above tracings correspond to numbers below nerve record, indicating the position of the animal at that point in the record. Top trace: time mark (100-ms intervals); second trace, RA2rt3s; third trace, RA3rt3s; fourth trace, RA4rt3s; bottom trace, photodiode signal of shutter movement. First tracing (0.45 s after light on) shows position of crayfish during hour in dark before light on (arrow) until shortly before second tracing at 5.4 s. Spikes were retouched.

under. The active units in root 2 were presumably TE motoneurons. Activity in both TF roots peaked at 9.2 s, when the animal began to flex the abdomen (frame 1, Fig. 2). The flexion was largely complete in 200 ms (frame 3), after which the firing frequency of all motoneurons gradually declined over several more seconds. The responses presented in Figs 1 and 2 were typical of those recorded in all trials from all six animals.

Responses of TF motoneurons to light directed at single abdominal ganglia

The responses described above indicate that extraretinal photoreceptors can excite **ostural** flexor and extensor motoneurons in the abdomen. To determine the location

of those photoreceptors, I stimulated individual abdominal ganglia with white and monochromatic green (546 nm) light. These experiments were performed on 50 animals in three preparations: isolated abdominal ventral nerve cords, isolated abdomens and intact animals. Two kinds of responses were seen: a slowly developing excitation of TFs that occurs in a number of ganglia and a strong, oscillating excitation of TFs that alternates with TE excitation. The slow, tonic TF response occurred in all three preparations while the bursting response was seen only in intact animals.

Fig. 3 presents responses of TF motoneurons, the caudal photoreceptor and other units in the A5–A6 connective to photostimulation of individual abdominal ganglia. These responses were recorded from an isolated abdomen preparation after 1 h of dark adaptation. Intervals of 15 min dark adaptation passed between stimuli. Stimuli delivered to ganglion A1 evoked no response (not shown), while stimulation of A2 and A3 evoked similar strong tonic responses from TF motoneurons in A3 and A4, and from unidentified units in the connective (Fig. 3, top two panels). Stimuli directed

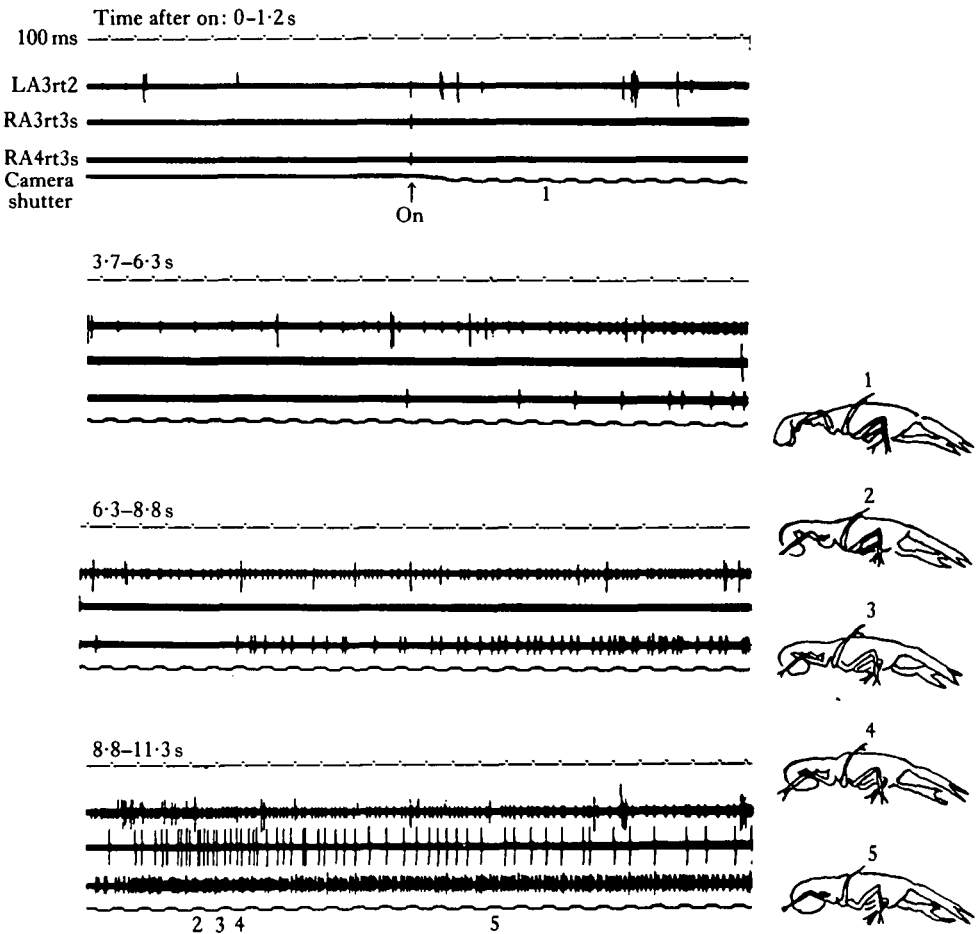


Fig. 2. Same situation as Fig. 1, with another animal. Activity of left 3rd abdominal segment tonic extensor motoneurons (LA3rt2) and right tonic flexor motoneurons (RA3rt3s) are displayed in the second and third traces, respectively. Activity of right TF motoneurons in 4th segment (RA4rt3s) displayed in 4th trace. Spikes were retouched.

at A4 evoked responses from motoneurons in A4, but not from those in A3 or from units in the connective (Fig. 3, third panel). Stimulation of A5 evoked only weak responses from TF motoneurons and units in the connective (Fig. 3, fourth panel). Stimulation of A6 evoked no response from TF motoneurons in A3 and A4, but evoked a strong discharge from the CPR (Fig. 3, bottom panel). The responses were repeatable, and not dependent on the order in which the ganglia were stimulated. Similar results were obtained by stimulating individual ganglia with monochromatic green (546 nm) light.

These responses are typical of those from all isolated abdomen or isolated abdominal nerve cord preparations. In general: the four small excitatory TF motoneurons

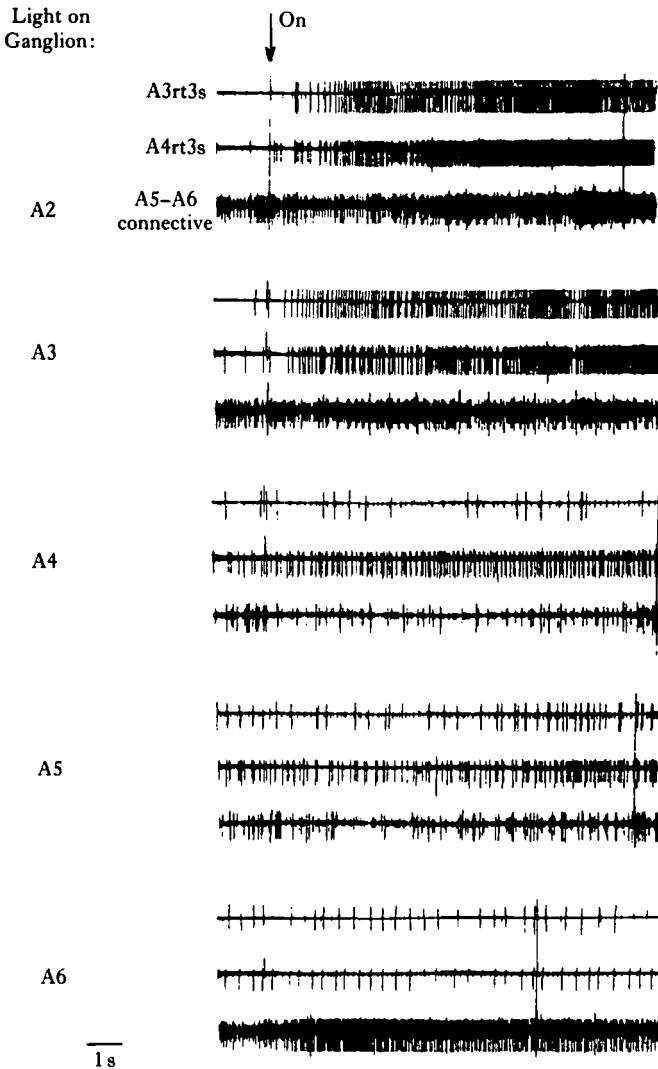


Fig. 3. Responses of TF motoneurons in A3 and A4, and of units in the A5-A6 connective to photostimulation of individual abdominal ganglia A2-A6, as indicated. The ventral nerve cord was cut between the abdomen and thorax. Arrow marks light on in each record. Large unit in third trace in bottom panel is the CPR response to A6 illumination. Spikes have been retouched.

in each hemisegment, f1–f4 (Kennedy & Takeda, 1965; Wine *et al.* 1974), respond to photostimulation of individual ganglia with a slowly increasing rate of firing) stimulation of A1 rarely evokes a TF response, while stimulation of A2, A3 and A4 usually evokes the largest TF responses; the responses usually are greater in the stimulated ganglion and in more caudal ganglia than in more rostral ganglia; the responses of TFs within each ganglion are bilaterally symmetrical; stimulation of more than one ganglion evokes more vigorous responses than are evoked by stimulation of single ganglia. Finally, although photostimulation of ganglia A2–A5 excites units in the abdominal connectives, the CPR is not among these. The CPR is excited by stimulation of A6, but this stimulus was never seen to evoke a TF response in rostral abdominal ganglia if the ventral cord was severed between the last thoracic and first abdominal ganglia. The lack of TF response to CPR stimulation suggested that the CPR plays no role in mediating TF excitation within the abdominal nervous system (Wilkins & Larimer, 1972).

When the ventral nerve cord was left intact, however, photostimulation of the last abdominal ganglion (A6) could evoke a strong, oscillating excitation from rostral ganglionic TFs. This is illustrated in Fig. 4, top panel, which presents the response of the CPR recorded in the connective, and the responses of TFs in A3 and A4. The response of the CPR had a latency of 1 s, and was characterized by an initial high-frequency burst of spikes, a low-frequency period immediately afterwards, and then a regular train of spikes that gradually slowed over many seconds, long after the light was off. The responses of the TFs in A3 and A4 began about 3 s after light on and peaked about 3 s later. At 9.5 s, the TF excitors (f1–4, f6) stopped firing for a period of 2.5 s, and then began another burst that lasted for 6 s more. This response long outlasted the period of photostimulation, which was 9 s. In other preparations, a strong discharge of the tonic flexor inhibitor motoneurone (f1) occurred during the interval between bursts of activity in the excitors.

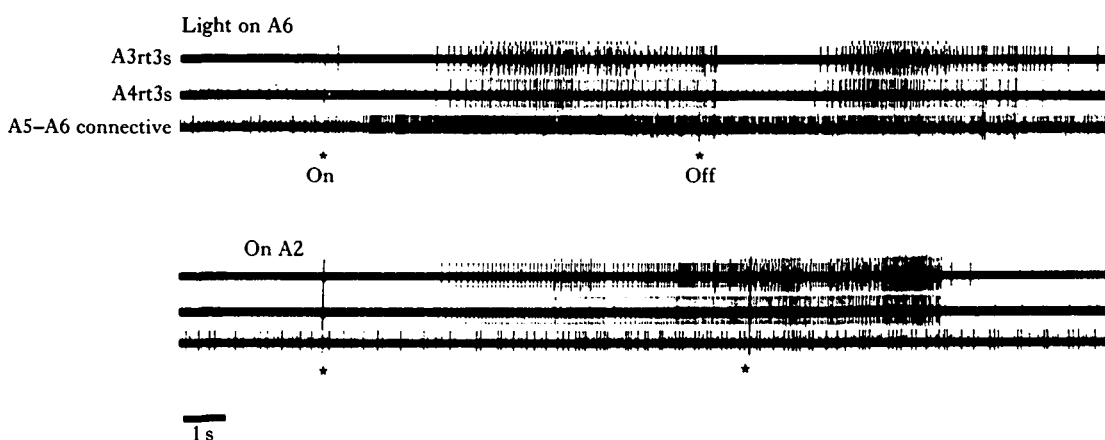


Fig. 4. Responses of TFs in A3 and A4, and of CPR in A5–A6 connective to photostimulation of individual abdominal ganglia. The ventral nerve cord was intact. Top panel: responses to stimulation of A6. Bottom panel: responses to stimulation of A2. Large unit in third trace of both panels is CPR. On and off of ganglionic illumination is indicated by asterisks.

The occurrence and latency of the TF discharge seems to depend on the frequency of the initial burst from the CPR. In Fig. 4, that frequency was over 100 Hz, and the latency of the TF response was 3 s; lower initial frequencies were associated with longer latencies and less vigorous responses. No responses occurred when the initial frequency of the CPR was below 80 Hz. The TF responses also failed to occur if the bundle of fibres that contained the CPR axon was cut in the connective between the abdomen and thorax. Neither photostimulation of A6 nor electrical stimulation of the CPR axon in the connective at 100 Hz evoked any response from the abdominal TFs under this condition.

On a very few occasions, light directed at abdominal ganglia other than A6 would evoke cyclical, bursting responses from TFs. An example of this from the same animal is presented in the bottom panel of Fig. 4, in which a bursting discharge from TFs in A3 and A4 resulted from photostimulation of A2. The response latency of the TFs was the same as for A6 stimulation, but the CPR increased its discharge rate only slightly above the resting value. Bursting responses were seen in response to photostimulation of any abdominal ganglion only if the ventral nerve cord was intact.

Responses of other motoneurons to photostimulation of single abdominal ganglia

In addition to the tonic flexor and tonic extensor motoneurons, swimmeret motoneurons are also affected by photostimulation. In Fig. 5, the intracellularly recorded response of a swimmeret motoneurone to ganglionic illumination is presented, along with extracellular responses of left and right ganglionic TFs. The swimmeret motoneurone, which was identified by its morphology (revealed by intracellular dye injection) and axonal projection out of the ganglionic first root, depolarized and increased its discharge rate concurrently with the TFs, at about 3.5 s after light on. Like the TFs, the swimmeret cell continued its discharge for some seconds after light off. Extracellular recordings from branches of first roots of abdominal ganglia have

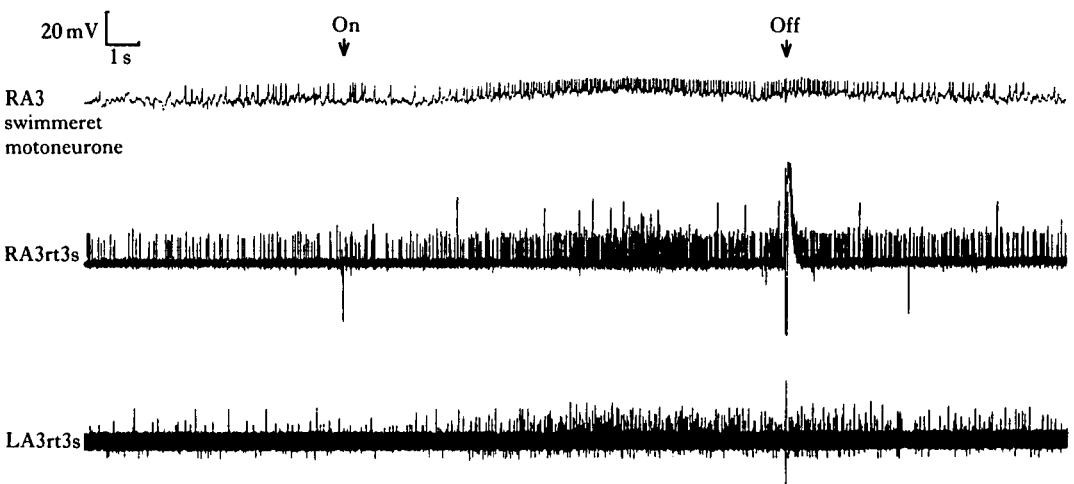


Fig. 5. Intracellularly recorded response of a swimmeret motoneurone to ganglionic photostimulation. The response of the cell to ganglionic illumination is presented in the top trace; the bottom two traces present the responses of TF motoneurons in the left and right superficial third roots.

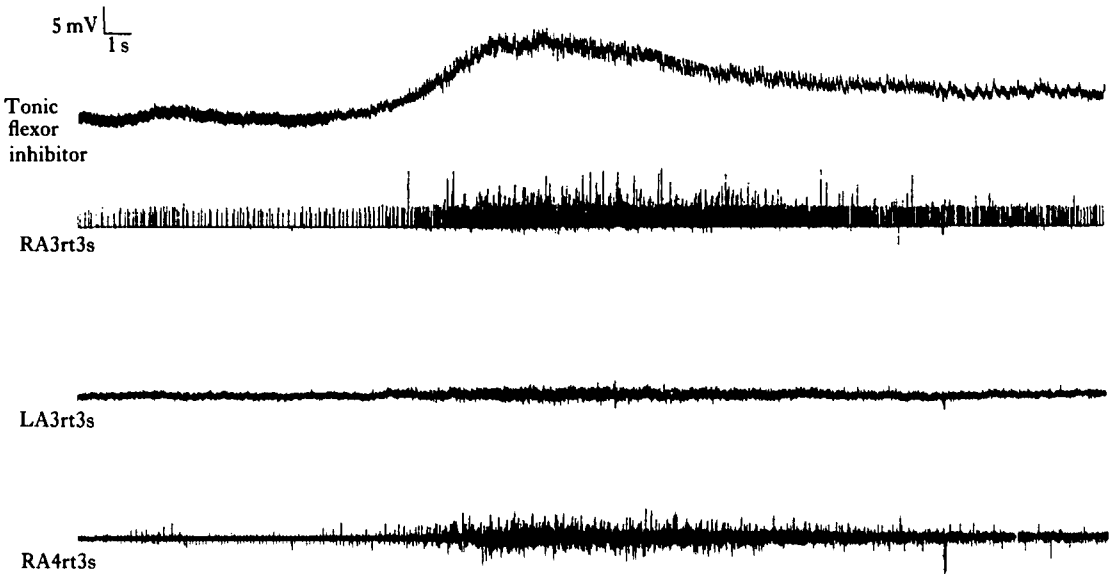


Fig. 6. Intracellularly recorded response of the tonic flexor inhibitor motoneurone to ganglionic illumination. The response of the cell to ganglionic (A3) illumination is presented in the top trace. The responses of TF motoneurones in the left and right superficial third roots of A3 and the right superficial third root of A4 are presented in the bottom three traces.

shown that ganglionic illumination causes swimmeret power stroke motoneurones to become excited and return stroke motoneurones to be inhibited (D. H. Paul, personal communication). This is consistent with the behavioural observation that swimmerets protract during the response to ventral illumination, and suggests that the swimmeret motoneurone of Fig. 5 is one of the set of power stroke cells.

The TF inhibitor motoneurone, which was also identified morphologically (Wine *et al.* 1974), rarely discharges in response to ganglionic photostimulation, but, as can be seen in Fig. 6, it does depolarize. The depolarization follows the increasing discharge in the other TF motoneurones, and reaches a peak of 12 mV above rest, recorded in the soma.

The fast flexor motoneurones are unaffected by photostimulation of the abdominal ventral nerve cord. The membrane potentials of both the fast flexor inhibitor and fast flexor excitor motoneurones were monitored from their somata during photostimulation, and no changes were observed.

DISCUSSION

Outputs of the caudal photoreceptors

At the time of their discovery (Prosser, 1934), the caudal photoreceptor neurones were implicated in the release of locomotor activity caused by illumination of the abdomen (Welsh, 1934). The results presented here indicate that the CPRs release a cyclical abdominal flexion-extension behaviour pattern that is connected with backwards walking. This behaviour pattern and the simple abdominal flexion that is

evoked by stimulation of rostral abdominal ganglia serve to remove the animal and its abdomen from brightly illuminated areas.

Fifty years after their discovery, it is still unclear where in the CNS the CPR neurones provide input to pre-motor systems. Both the work of Wilkens & Larimer (1972) and the data presented here indicate that the CPRs make no output connections in the abdomen, and so it can be assumed that the cells mediate locomotor activity through connections in the thoracic and/or more anterior ganglia. The results of the behavioural and electrophysiological experiments described above suggest that those connections might include one or more 'backward walking' command fibres (Kennedy, Evoy, Dane & Hanawalt, 1967; Kovac, 1974*a,b*; Bowerman & Larimer, 1974). This suggestion is made because the cyclical pattern of TF activity released by CPR stimulation (Fig. 4) can also be released by repetitive electrical stimulation of a backward walking command fibre in the abdominal ventral nerve cord, and by repetitive electrical stimulation of root 5 of A6 at 36 Hz (Kovac, 1974*a*). Stimulation of this same root at this frequency has also been found to drive the ipsilateral CPR one-for-one (Wilkens & Larimer, 1972). Considered together, these data suggest that the CPR neurones may act to excite the backward walking command fibre(s) at some site in the CNS rostral to the abdomen. This suggestion is consistent with the behavioural observation that backward walking frequently accompanies flexion evoked by ventral illumination of blinded animals, and with the finding that other inputs to backward walking command units occur in the rostral portion of the CNS (Bowerman & Larimer, 1974; Kovac, 1974*a,b*).

The results presented here may also provide some insight into how the backward walking command system is activated by the CPRs. First, considerable temporal summation of CPR inputs appears to be necessary to activate the command system. This is suggested by the observation that abdominal motor output is not released by CPR stimulation unless the frequency of the cell's initial burst of impulses is above 80 s^{-1} . If temporal summation is necessary to activate the command system, then faster temporal summation should activate it more rapidly; this may account for the observation that the latency of that motor output is shorter for higher initial CPR burst frequencies. Second, after its activation by the initial CPR burst, the command system appears to become relatively independent of CPR input. This is suggested by the persistence of the oscillatory motoneuronal response long after the initial high-frequency CPR burst that triggers it or the period of the illumination of A6 (Fig. 4). Both of these suggestions are supported by the early observations of Welsh (1934), who found that leg motion continued for several seconds after the light stimulus of A6 was cut off. They are also consistent with the observation here that in freely behaving animals, the initial flexion of the abdomen in response to ventral illumination greatly reduces the intensity of light reaching the 6th ganglion, and yet the backward walking behaviour pattern and concurrent partial re-extensions and flexions (when they occur) persist for many more seconds.

Outputs of other abdominal extraretinal photoreceptors

Illumination of individual abdominal ganglia other than A6 tonically excites both tonic flexor and power stroke swimmeret motoneurones (Figs 3, 5). The tonic flexor response is bilateral and extends to ganglia caudal to the illuminated ganglion; the

response of rostral motoneurons is usually weak or absent (Fig. 3). These same motoneuronal responses are observed in isolated abdominal nerve cords and intact animals which indicates that, unlike the TF activity released by the CPRs, these motor responses are the result of local neural interactions within the abdominal nervous system.

The spread of TF excitation to ganglia caudal to the illuminated ganglion suggests that illumination of more than one ganglion will evoke a summed response from TF motoneurons receiving excitation from more than one source. That this is the case is indicated by the observation that illumination of two or more ganglia releases a much more vigorous TF response than illumination of any single ganglion. This suggestion may also account for the observation that the TF responses of the implanted animals (Figs 1, 2) reached a peak of activity in more caudal ganglia earlier than in more rostral ganglia. The rostrally progressing wave of TF activity allows the abdominal flexion to proceed smoothly from caudal to rostral segments.

The caudally directed activation of TF motoneurons is not unique to light-activated responses, but rather reflects the underlying intersegmental connections of the abdominal postural system. TF motoneurons in rostral segments have higher spontaneous discharge rates than those in more caudal ganglia (Evoy *et al.* 1967), and TF responses to afferent inputs are greater in caudal ganglia, regardless of which segment receives the input (Tatton & Sokolove, 1975a). The activation of these interganglionic interconnections may account for the large amount of activity recorded in the 5-6 connective during illumination of individual rostral ganglia (Fig. 3).

The identity of photoreceptors in rostral abdominal ganglia

Unlike the CPRs, whose morphology and region of photosensitivity in A6 has been described (Wilkins & Larimer, 1972), the identity of those photoreceptors that release the motor responses described here remains unknown. Positive identification of those transducer cells may be difficult with electrophysiological techniques alone. This difficulty arises because the intracellularly recorded responses of the swimmeret motoneurone (Fig. 5) and the tonic flexor inhibitor motoneurone (Fig. 6) both exhibit slow depolarizations in response to illumination that are similar to that recorded in the CPR in response to illumination of A6 (Kennedy, 1963; Wilkins & Larimer, 1972). This similarity could suggest that these cells are primary photoreceptors were it not that their identity as motoneurons makes it impossible for them to mediate individually the diverse responses released by light (Tatton & Sokolove, 1975b; D. H. Paul & V. McDonald, personal communication). Since it is likely that a pre-motor interneurone would also respond to ganglionic illumination with a slow depolarization, such a cell might easily be mistaken for a photoreceptor on the basis of its response to light and its ability to release the appropriate set of motoneuronal responses when driven electrically. These difficulties might be alleviated with the use of immunocytochemical techniques to identify cells in the abdominal ganglia that contain rhodopsin-like substances (Bruno & Kennedy, 1963; Beltz & Kravitz, 1983).

Extraretinal inputs, visual inputs and behaviour

It is clear from the results of the behavioural experiments described here that abdominal extraretinal photoreceptors, including the CPRs, can mediate backward

walking and/or tail flexion responses to abdominal illumination. It is also clear that visual inputs can increase the probability of response, and decrease the response latency and duration of flexion (Tables 1, 2). Visual inputs alone can also produce locomotor activity (Welsh, 1934), including backward walking away from illuminated parts of the environment (Kovac, 1974a). From these results, we can conclude that the crayfish is equipped with at least three photosensory systems that ensure that the animal removes itself from strong illumination. The visual system senses illumination of the anterior end of the animal, while the CPRs sense illumination of the posterior end; they both cause the animal to retreat out of the illumination. The photoreceptors in the anterior abdominal ganglia also sense when the abdomen is illuminated, but release a slow abdominal flexion alone without backward walking. This suggests that this system is invoked when the anterior end of the animal is already under cover, so that a slow flexion can slowly withdraw the tail from view without moving, and possibly exposing, the whole animal.

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