J. exp. Biol. **164**, 153–169 (1992)
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SENSORY INPUTS MEDIATING TWO OPPOSITE BEHAVIOURAL RESPONSES TO LIGHT IN THE CRAYFISH *PROCAMBARUS CLARKII*

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Accepted 28 November 1991

Summary

Crustaceans are attracted out of their burrows by dim light whereas they withdraw in response to light of high intensity. We have studied attraction and withdrawal responses in freely walking crayfish (*Procambarus clarkii*) and in animals tethered above a treadmill device. Electrophysiological recordings have been made of retinal and extraretinal visual units to determine their possible inputs.

Attraction was induced by light of 0.17–1.4 lx; the crayfish walked forward with the abdomen extended. Withdrawal was observed at intensities above 5.6 lx; the crayfish walked backwards with a cyclic pattern of flexion and extension of the tail. A group of sustaining visual neurones of the optic nerve with low thresholds was found to respond to light in the range of intensities that produced attraction. Two groups of neurones responded in the range of intensities that produced withdrawal: sustaining visual neurones with high thresholds and the caudal photoreceptors. In animals in which the visual fields of the high-threshold fibres had been covered with black paint, the attraction response was not affected, while withdrawal was only observed in response to light stronger than 11 lx. When tested on a treadmill, the latency of the responses to light and the direction of walking of crayfish reflected attraction and withdrawal responses. Moreover, under these conditions, illumination of the eye induced forward walking at intensities that produce attraction and backward walking at intensities that produce withdrawal. In contrast, illumination of the tail induced only backward walking.

Introduction

In their natural environment, animals respond to different sources of sensory stimulation. The decision to initiate an appropriate behavioural movement is the result of complex neurophysiological integration.

Field observations of crustaceans have revealed a burrow-emergence behaviour

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Key words: crayfish, behaviour, vision, sensory integration, *Procambarus clarkii*.

at dawn and dusk, while the animals remain hidden during periods of full light or complete darkness (O'Riordan, 1964; Andersen, 1962; Hillis, 1971*a*,*b*). By combining field studies with laboratory observations, it has been shown that dim lights attract the animals out of their burrows, while high intensities induce them to withdraw (Aréchiga and Atkinson, 1975). Little is known about the range of light intensity that produces each of the responses and, although the visual system of the crayfish has been widely studied, the elements that participate in attraction and withdrawal responses and what determines the choice of response remain unknown.

In the crayfish *Procambarus clarkii*, responses to light intensities in the optic nerve are mediated by 14 visual neurones, known as sustaining fibres (SFs). These neurones discharge in a phasic–tonic manner at a frequency proportional to the light intensity and with a duration equal to that of the stimulus (Wiersma and Yamaguchi, 1966; Glantz, 1972). Each of the 14 neurones of the group can be distinguished by its defined sensory field in the retina (Wiersma and Yamaguchi, 1966). Their somata are localised in the internal medulla (Kirk *et al.* 1982) and their axons run through the optic nerve to the cerebral ganglion, where they establish connections with descending visual interneurones (Wood and Glantz, 1980*a*) and with local interneurones (Okada and Yamaguchi, 1988). A second type of neurone responding to light intensity is the caudal photoreceptor (CPR). Two of these neurones are located in the sixth abdominal ganglion (Prosser, 1934; Kennedy, 1958, 1963; Wilkens and Larimer, 1972), and they respond to bright light with an increase in their firing frequency (Kennedy, 1958, 1963; Wilkens and Larimer, 1972). It is known that intense light directed onto the tail of the crayfish induces locomotor activity (Prosser, 1934; Welsh, 1934) and Edwards (1984) has found that illumination of the tail produces tail flexion followed by backward walking. In addition, intracellular stimulation of the CPRs induces excitation of the command neurones for walking and also induces leg movements (Simon and Edwards, 1990).

The aim of this work was to reproduce and characterise, in the laboratory, attraction and withdrawal responses in the crayfish *Procambarus clarkii* and to study the possible participation of the SFs and the CPRs.

Materials and methods

Adult *Procambarus clarkii* of either sex were used at the intermoult stage. Prior to the experiments, crayfish were kept for at least 1 week in individual compartments with a simulated burrow. During this period, animals experienced controlled 12 h:12 h light:dark cycles and were fed weekly.

Behavioural experiments

The system for the quantitative analysis of behaviour consisted of five two-compartment chambers (actographic chambers) made of black Perspex. One of the compartments was a long slender rectangular tunnel simulating the shape of a

natural burrow (Fernández-de-Miguel *et al.* 1989). This opened into a wide chamber, allowing attraction and withdrawal responses to occur. To detect locomotor activity, each compartment was fitted with five pairs of infrared photocouples connected in parallel to a circuit that generated a pulse every time the animal crossed one of the light beams. The photodiode (TIL 31) had a wavelengh of 940 nm, which was out of the range of spectral sensitivity reported for crustacean photoreceptors. Each of the channels was connected to a microcomputer (Commodore 16) through a digital interface. A computer program identified the source of information and a classification key was assigned. When the lights were switched on or off, a keyboard signal was sent, registering the chamber number and the light intensity. Information was stored on disk and a real-time list of events was generated which included the following: the number of the recording chamber in which the event occurred; the time; the latency of change from one compartment to another; the number of movements in each compartment prior to the change; a comment on light intensity; whether lights were turned on or off; and any change of compartment.

The light source consisted of a white light bulb placed above the chamber and controlled by a calibrated potentiometer to give a range of light intensities.

Chambers were placed in a room at constant temperature (23 ± 1 °C) and isolated from noise. Crayfish were placed in the chambers for at least 5 days before the experiments and their spontaneous locomotor activity was recorded. Once the animals showed a nocturnal locomotor activity rhythm similar to that described for this species (Page and Larimer, 1972; F. Fernández-de-Miguel and H. Aréchiga, in preparation), attraction and withdrawal responses were tested. Crayfish that did not show burrow-emergence behaviour or had low levels of spontaneous activity were discarded.

Tests were carried out at the beginning of dark periods and during periods of high spontaneous activity on animals adapted to dark for at least 2 h. Every animal started its period of spontaneous locomotion at a different time and so, to decrease variability, the activity of a crayfish was continuously monitored and the series of trials was initiated after the animal had started its peak of locomotor activity. Some animals with low levels of spontaneous locomotor activity were found. These animals characteristically remained in their burrows for long periods and showed almost no responsiveness to light. This was observed particularly 1 or 2 days after the crayfish had been fed so only animals fed 1 week before the experiments were used. Nevertheless, at the beginning and end of the peak of spontaneous locomotor activity, the latency for walking out of the burrow tended to increase. For this reason, the length of the tests was restricted to about 2.5 h, starting 30 min after locomotor activity had increased. This drastically reduced the variability in the latencies of spontaneous changes of compartment in darkness and of the responses to light, both in the behaviour of a single animal and between one animal and another. In addition, the decrease in variability allowed us to group the data from different animals instead of treating them separately, allowing further comparisons of the behaviour with neuronal responses.

Tests consisted of turning on the light in the open space and monitoring the latency of the animal's change of compartment at every intensity. Attraction responses occurred when the animal was initially inside the burrow and came out following illumination. Withdrawal occurred when the animal was in the open space and walked back into the burrow as a result of illumination. When a change was detected, the lights were turned off and the crayfish was allowed to re-adapt to darkness for 20 min. Latencies shorter than the mean time ± standard error (S.E.) of spontaneous changes of compartment in darkness were considered to be produced by the light stimulus. One or two days after the first test session had taken place, the same animals were tested again to increase the number of observations per animal. Data were expressed as the latency *versus* the logarithm of the light intensity (latency/log I relationship).

Recordings of the sustaining fibres

Electrical recordings of the SFs were made by inserting a polished stainless-steel electrode into the optic nerve of the crayfish to localise and identify the SFs. Unitary activity was then recorded using electrode tips between 1 and 10 μm in diameter (see Wiersma and Yamaguchi, 1966). With bigger tips, multiunit responses could be measured.

Before recording, the animals were fixed by the carapace to restrict movement. For electrode insertion, the rostrum was removed at least 1 day before the experiments and the eyestalk was glued to the carapace to prevent eye movement.

Signals were received by an a.c. preamplifier (Grass P-15), an oscilloscope and an audio monitor and were stored on tape. A window discriminator was used to determine the individual frequencies in multiunit recordings.

Before recordings were made, animals were dark-adapted for 1 h, and stable thresholds (one spike per light pulse) were obtained during four consecutive pulses with 5 min intervals between them. A calibrated monochromatic (414 nm) light-emitting diode (LED) was used as a light source. Intensities up to 170 lx were obtained when fed through a current source.

SFs could be identified by their responsiveness to light when the different sensory fields were illuminated by an optic fibre. Firing frequencies were measured at different intensities after 1 s of illumination to avoid the phasic component of the response and adaptation of the neurone. Results were expressed as the frequency *versus* the logarithm of light intensity (F/log I).

Recordings from the caudal photoreceptors

Activity of the CPRs was recorded from isolated preparations. The abdominal chain was first dissected out and the sixth abdominal ganglion isolated. The connective nerves were separated into small bundles of axons and tested with a suction electrode until CPR activity was found. Electrical recordings of dark-adapted animals were carried out using the same procedure described above for the SFs. The spontaneous activity of the CPR and the background activity of other units present in the recordings were subtracted from the records to give the firing

frequency of the CPR during illumination. Results were expressed as an $F/\log I$ relationship.

Experiments with painted retinas

Tests were made on crayfish before and after the visual fields of the high-threshold SFs had been covered. The eyes of the animals were dried and three layers of black water-soluble, non-toxic paint were applied to the sensory fields of neurones O30, O38, O14 and O9 (See Wiersma and Yamaguchi, 1966). When the paint was dry, it was covered with a layer of transparent nail polish. Using this procedure, the sensory fields of the SFs were isolated from light for several days. Before painting the sensory fields, the behavioural responses of the animals were recorded as their own controls.

Treadmill experiments

To correlate different variables, such as light intensity, behavioural latencies, the direction of walking and the activity of muscular elements, a treadmill was designed and constructed. The crayfish was tethered by means of a cork glued to the carapace (Fig. 1). When the animal walked, the displacement of the treadmill band was in the opposite direction to that of walking. The band was coupled to a movement detection system. This consisted of a hexagon with a hole in each of its angles and an infrared photocouple system. Light emitted from a photodiode (TIL31) was passed through the holes and received on the other side by a

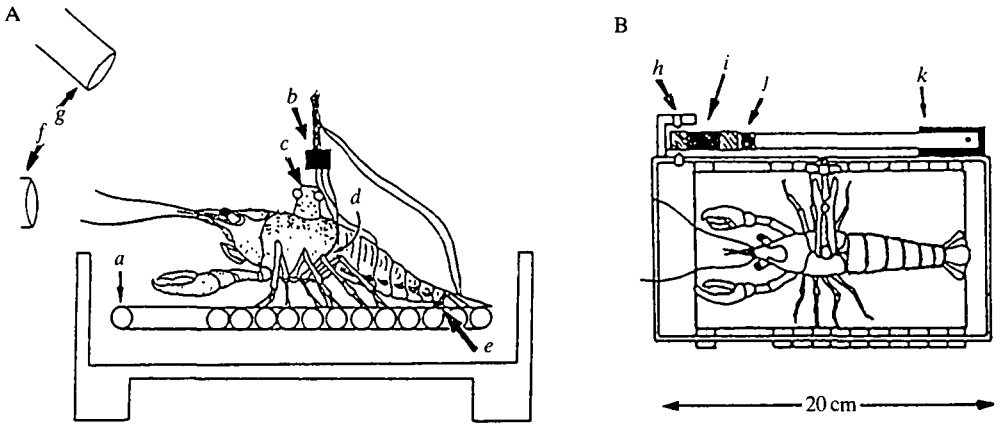

Fig. 1. (A) Lateral view of the treadmill used for movement detection. *a*, cylinder to activate the hexagon; *b*, connector for recording electrodes and tail-light source; *c*, cork glued to the dorsal carapace for fixing the animal; *d*, electrode for recording the activity of the merocarpopodite articulation; *e*, source of light for tail illumination; *f* and *g*, diffuse light sources. (B) Top view of the treadmill. *h*, photocouple system for motion detection; *i*, hexagon; *j*, microswitch activator; *k*, microswitch.

photoreceptor (TIL 38). Every time the light crossed the hexagon, a pulse was generated.

The output from the circuit was connected to a two-step microswitch also activated by the hexagon. When the crayfish walked forward, the hexagon turned the switch to the 'down' position and the output to the pulses generated by the photocoupling system was through channel 1. In contrast, when the crayfish walked backwards, the hexagon turned the switch to the 'up' position and the photocouple output was through channel 2. Output signals were recorded in such a way that when the animal walked forwards or backwards the pulses were in the upward or downward direction, respectively (see Fig. 6).

Three different sources of calibrated light were used in the same experiment (see Fig. 1). The first was placed 10 cm in front of the animal to simulate illumination inside the burrow (*f* in Fig. 1); the second was 10 cm above the animal to reproduce the conditions encountered when out of the burrow (*g* in Fig. 1). The third was a light-emitting diode (LED) fed through a current source with a thin wire and fixed to the ventral part of the abdomen under the sixth abdominal ganglion (*e* in Fig. 1). In this way, the crayfish was kept without restrictions on tail movement and the CPRs were illuminated without the retina being affected.

Recordings of junction potentials

During walking, junction potentials of the flexor muscles of the merocarpopodite articulation (MCA) of the third walking leg were recorded since it participates in forward and in backward walking and does not seem to be specialised for other tasks (Ayers and Davies, 1977). A stainless steel-electrode inserted and glued into the MCA (Figs 1, 6) was connected to an a.c. preamplifier (Grass P15). Activity was recorded on tape and on paper simultaneously for analysis.

Results

Behavioural experiments

Attraction and withdrawal behaviour were induced in crayfish under laboratory conditions. After 1 week under 12 h:12 h light:dark cycles, most of the spontaneous burrow-emergence behaviour occurred during the first 3 h of darkness (F. Fernández-de-Miguel and H. Aréchiga, in preparation). Some animals showed much lower levels of spontaneous activity than others; such animals were excluded from the experiments and only animals expressing a locomotor activity rhythm that included burrow-emergence during the first hours of darkness were tested. Under these conditions, the mean time that a group of 12 crayfish stayed inside their burrows without light stimulation was 6.6±1.4 min (±S.E., N=32), while they stayed outside their burrows for 7.2±1.5 min. These values were compared with the latencies for attraction and withdrawal responses, characterised by latencies smaller than the average for a spontaneous change of compartment (Fig. 2C,D).

When a dim light was turned on outside the burrow, the attraction response was

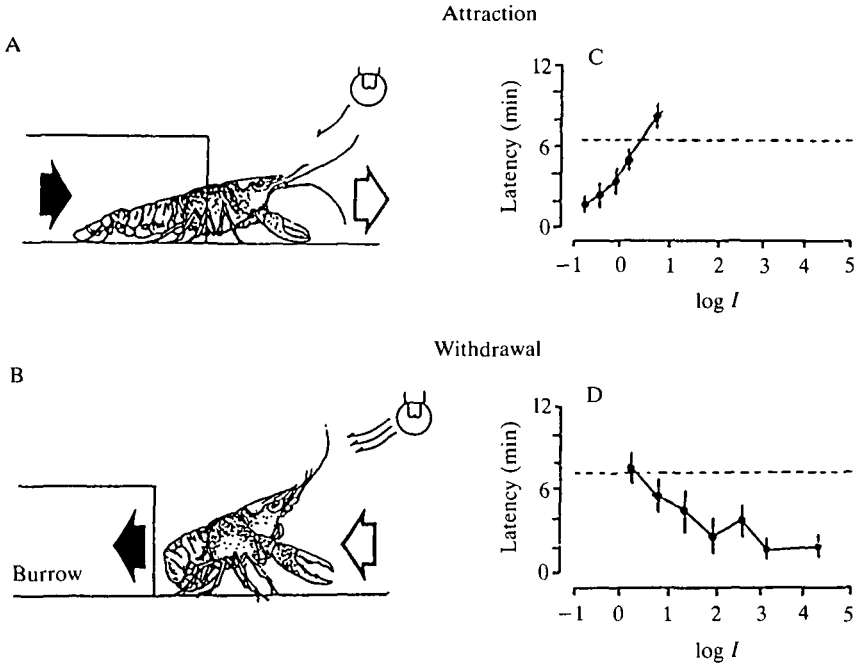

Fig. 2. Schematic representations of (A) attraction and (B) withdrawal responses. During attraction the crayfish walks forwards with its abdomen extended, while during withdrawal it walks backwards with cyclic flexions of the tail. (C, D) Plots of latency (min) *versus* the logarithm of light intensity (lx) for attraction (C) and withdrawal (D). The spontaneous time for a change of compartment is indicated by a discontinuous line on each graph. Values are mean±S.E., obtained from a group of nine animals.

induced. Average responses (±S.E.) from nine animals, each tested several times, are presented in Fig. 2C. Attraction was observed between 0.17 and 1.4 lx. At 0.17 lx animals responded in 54 % of the trials (N=28), whereas no responses were detected at lower intensities. The shortest latencies were recorded at 0.17 lx (1.8±0.6 min). The latency increased with increasing light intensity. At 1.4 lx, latency was 5.48±0.4 min (N=15), similar to that of spontaneous emergence from the burrow (6.22±0.45 min). Above this intensity, attraction latencies continued to increase and the response disappeared above 22 lx. Visual inspection of the animals showed that during the attraction response they always came out of their burrows in a stereotyped way, walking forwards with the abdomen extended (Fig. 2A).

Withdrawal behaviour occurred at light levels above 2.8 lx. At this intensity, latencies (7.49±1.1 min, N=18) were similar to that for a spontaneous change of compartment (7.11±0.94 min); the latency decreased with increasing light intensity. The latency/log I relationship for withdrawal had two components. The first occurred between 2.8 and 88 lx and consisted of a decrease in the latency as the intensity of light was increased. The second was observed at intensities over 88 lx;

between 88 and 1000 lx no significant differences were observed in the latencies (Fig. 2D). The lowest latency for withdrawal was found to be 1.7±0.8 min (N=21) at 1050 lx. Withdrawal was also a highly stereotyped behaviour pattern consisting of backward walking accompanied by cyclic flexion and extension of the abdomen (Fig. 2B).

Responses of the sustaining fibres

To implicate a given neurone in a behavioural pattern it is necessary to show that its range of responsiveness is similar to that of the behaviour pattern. Consequently, a comprehensive study was performed on different SFs to compare their dynamic ranges with those of the behavioural responses.

In an attempt to reproduce behavioural conditions, the eye of an animal whose movements were restricted was illuminated with diffuse light. Of the 14 SFs making up the group, 12 were found and studied on at least two occasions in different animals. The visual field of each neurone was identified by passing a 50 μm diameter optic fibre through the eye while recording SF activity. All the SFs had dynamic ranges of between 1.5 and 1.8 logarithmic units (Glantz, 1972). Owing to the reproducibility of their dynamic ranges, the thresholds of SFs are used in Table 1 as an expression of their sensitivity. Thresholds were found in a range between 0.016 lx (for O20) and 0.38 lx (for O38).

Table 1. *Threshold responses (median values±range) of the sustaining fibres to eye illumination with diffuse light*

Neurone	Threshold (lx)	Range (lx)	N
Low-threshold fibres			
O20	0.016	0.004	3
O72	0.028, 0.030		2
O19	0.030	0.006	4
O74	0.036, 0.048		2
O56	0.048	0.008	3
O16	0.046, 0.054		2
O2	0.052	0.005	5
O22	0.070, 0.702		2
High-threshold fibres			
O14	0.094	0.082	3
O30	0.156, 0.184		2
O9	0.246, 0.054		2
O38	0.38	0.078	6

Neurones were classified as low-threshold or high-threshold on the basis of the percentage of their dynamic range that fell within attraction (low-threshold) or withdrawal (high-threshold).

In those cases in which only two neurones of one type were recorded, the individual values of the thresholds are shown.

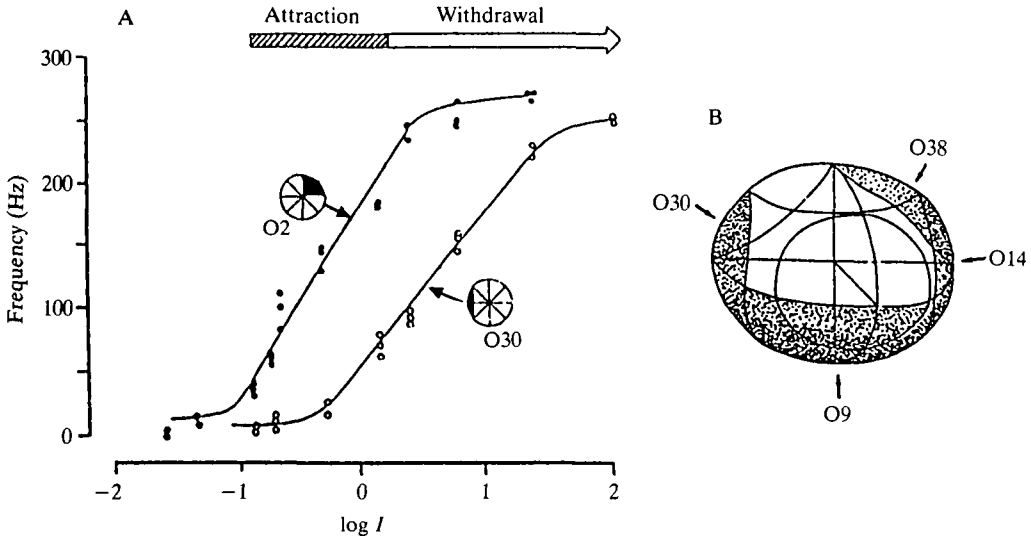

Fig. 3. (A) Frequency *versus* the logarithm of intensity (lx) for two sustaining fibres. O2 is classified as low-threshold, while O30 is in the group with high thresholds. Sensory fields are shown with each curve. Each point represents a different observation. The bars at the top indicate the light intensity at which each of the behavioural responses was produced. (B) Schematic representation of the right eye of the crayfish. The continuous lines indicate the sensory fields found by Wiersma and Yamaguchi (1966). The stippled zones indicate the visual fields for the sustaining fibres with high thresholds. The sensory fields of the eight low-threshold SFs cover the whole surface of the eye.

Eight of the SFs studied responded (over 80 % of their dynamic range) to intensities that induced attraction. The remaining four responded to intensities that produced withdrawal. Fig. 3A compares the responses of two SFs and the light intensities for attraction and withdrawal. SF O2 had a dynamic range between 0.08 and 2.8 lx, with attraction occurring between 0.17 and 1.4 lx. At 2.8 lx, O2 fired at 230 Hz (mean value, N=5) which is close to saturation. SF O30 had a different dynamic range. At 0.7 lx it entered into its dynamic range and at approximately 50 lx it reached saturation. As in Fig. 2B, withdrawal started to be observed above 2.4 lx. At this intensity, O30 fired at an average frequency of 76 Hz and had covered only 28 % of its dynamic range. Fig. 3B shows the visual fields of SFs (adapted from Wiersma and Yamaguchi, 1966); the visual fields of the high-threshold SFs are stippled. They are mainly located at the periphery of the eye, while the sensory fields of the low-threshold SFs overlap and cover the surface.

When the eye was stimulated with diffuse light, a bursting pattern in which several neurones were synchronised was observed (Waldrop and Glantz, 1985). We observed this behaviour between 0.1 and 2.8 lx. Fig. 4 shows the synchronised activity of four fibres firing in bursts. The bursting response occurred within the range of light intensities that produced attraction. The response of O2 at 1 lx is

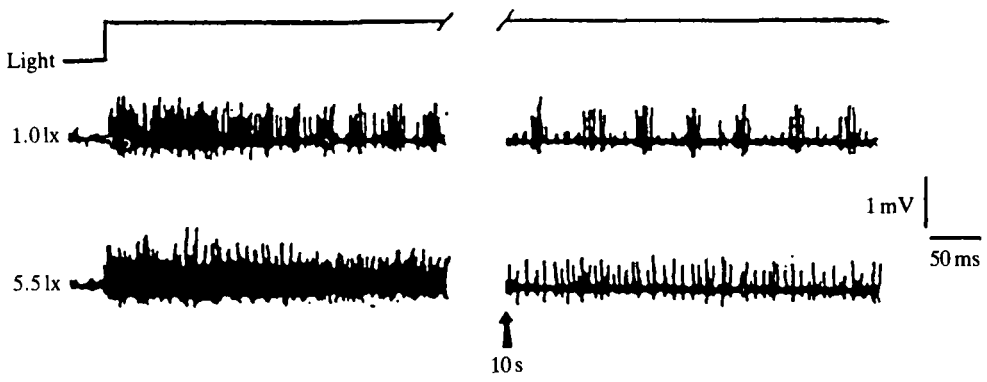

Fig. 4. Multiunit recordings of the bursting response of sustaining fibres O2, O19, O21 and O38 to light levels of 1.0 lx, which is in the range of attraction, and 5.5 lx, which produces withdrawal. Recordings are shown from time zero after the beginning of the light stimulus and after 10 s. At 1.0 lx, bursting behaviour can be observed during the tonic response even after more than 10 s. At 5.5 lx, the bursting response is desynchronized. The top trace shows the beginning of the light pulse.

again used to explain this phenomenon. In the interval between 1000 and 1050 ms of illumination, the median firing frequency of the five different neurones was 140.8 Hz; however, when single bursts in the same recordings were analysed, the median firing frequency within the burst was 354.6 Hz. Burst duration depended on two factors: light intensity and pulse duration. After 300 ms at 1 lx, bursts of O2 lasted 26.75±1.3 ms (±S.E.), while after 10 s the bursts lasted 19.5±1.1 ms. Above 2.8 lx the bursting response of the SFs was desynchronised, although the average firing frequency of the SFs was unaffected.

Responses of the caudal photoreceptor (CPR)

The responses to light of the CPR have already been described (Kennedy, 1958, 1963). To compare the dynamic range of the CPR and the intensities of light that produce attraction and withdrawal responses, we recorded the responses to light of six CPRs from isolated preparations. Under our experimental conditions, the threshold of the response to light was found to be 50 lx. Above 150 lx, the increase in firing frequency was linear and proportional to the logarithm of the light intensity (Kennedy, 1963). This behaviour was maintained up to 1500 lx (the highest intensity tested). The time to reach a steady-state response during a light pulse was also dependent on light intensity. At 200 lx, the steady-state response was obtained after 6.5 s, while at 1000 lx the delay was 2.7 s.

Comparing the dynamic range of the CPR with those of the behavioural responses, it was found that withdrawal starts at approximately one logarithmic unit below the threshold of the CPR. The dynamic range of the CPR included high intensities of light that produced a withdrawal response in the animal. Moreover,

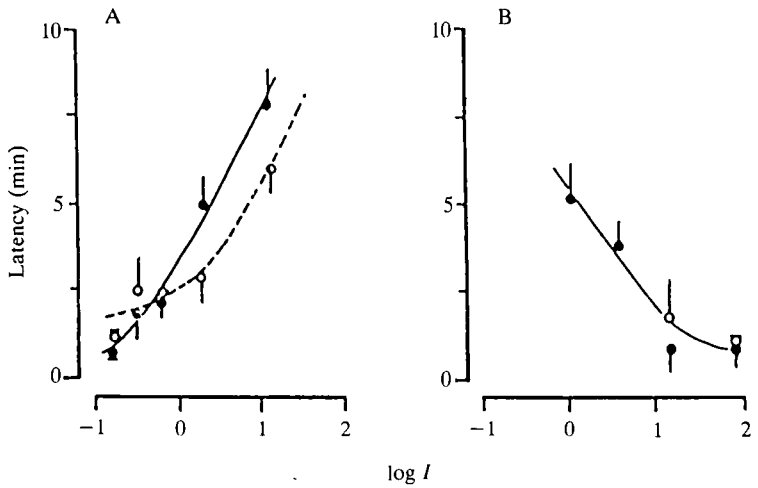

Fig. 5. Effect of painting the visual fields of the high-threshold SFs on the latency/log I relationships for (A) attraction and (B) withdrawal responses. The filled circles are the controls before the visual fields had been painted; the open circles represent the latencies after the visual fields had been painted. No withdrawal response was observed below 11 lx in animals with painted visual fields; values are means with standard errors in each case (N=6). Light intensity is measured in lx.

at these intensities the SFs were saturated, suggesting a possible role for the CPR in withdrawal behaviour at high intensities.

Behavioural effect of selective illumination of the visual fields of the low-threshold sustaining fibres

The visual fields of the low-threshold SFs cover the surface of the whole eye, but those of the high-threshold SFs are distributed around the periphery (see Fig. 3B). To study the contribution of the two different groups of SFs to attraction and withdrawal responses, the visual fields of neurones O30, O38, O14 and O9 were covered with black paint in six animals. The same animals were tested before the visual fields were painted.

The latency/log I relationship for attraction behaviour (N=6) showed two differences compared with controls. For intensities between 0.14 and 0.7 lx no change was observed in the latency of attraction (Fig. 5A). At higher intensities, the curve was shifted to the right. This caused a significant decrease ($P<0.05$, Student's t-test) in the latency at 2.8 lx. In controls, 2.8 lx induced attraction after 5.1±0.8 min (±S.E., N=14), whereas after the visual fields had been covered, the latency was 2.9±0.7 min.

Withdrawal behaviour was also modified. After the visual fields of the high-threshold SFs had been covered, withdrawal was only observed at intensities above 11 lx (Fig. 5B). Latencies before and after the retinas had been painted were very similar to those in the controls. The latency at 11 lx was 1.78±1.29 min (N=6) whereas at 88 lx it was 1.22±0.55 min.

Effects of illumination on the activity of the merocarpopodite articulation

The latencies of the responses to illumination and the direction of walking under different conditions of light stimulation were studied in eight animals tethered over a treadmill device (Figs 1, 6). A total of 156 tests were made applying light intensities between 0.17 and 300 lx. Intensities between 0.17 and 1.4 lx induced

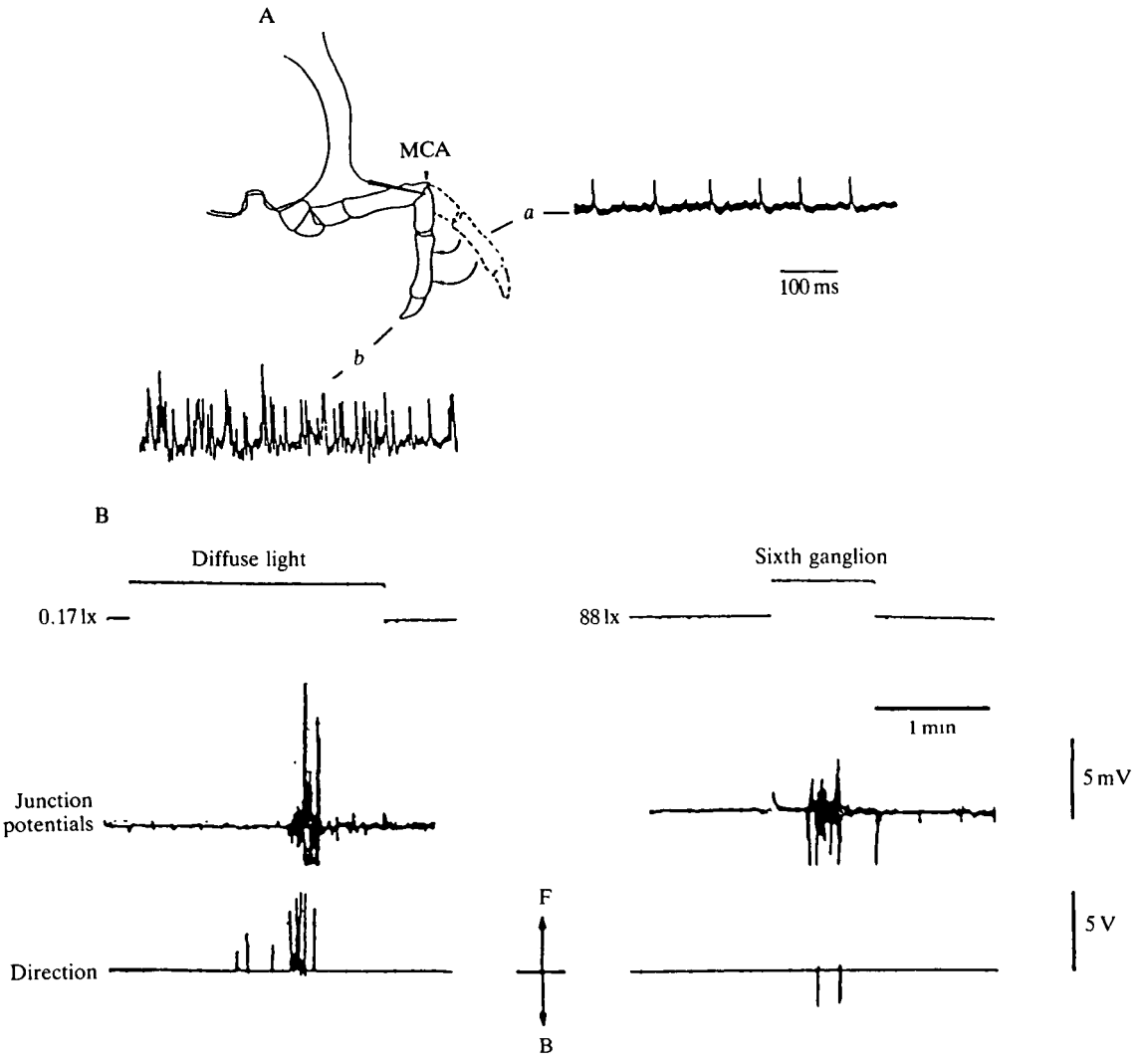

Fig. 6. (A) Diagram of the recording technique and electrical recordings of junction potentials of the merocarpopodite articulation (MCA) (*a*) before and (*b*) during movement. (B) Recordings of junction potentials and the direction of walking under different conditions of illumination from crayfish tested on the treadmill device. The top trace represents the pulse of light; F and B indicate forward and backward walking, respectively.

forward walking in 76.5 % of the tests. In 10.2 % of the cases no movement was recorded and in the remaining 13.3 % backward walking was detected. Between 2.8 and 300 lx, backward walking occurred in 83.4 % of the tests. In 5.7 % of the tests forward walking was recorded and in the remaining 10.9 % no movement was detected.

Spontaneous walking was also detected. In 58.3 % of the cases it was forward while in 21.6 % it was backward. In 20.1 % of the cases, activity in the merocarpopodite articulation (MCA) was not followed by walking. The intervals between periods of spontaneous walking were 3.88±0.11 min and the duration of locomotion was 1.52±0.15 min.

Recordings of the junction potentials from the merocarpopodite articulation of six animals showed that, at rest, junction potentials of around 10 ms duration were generated with a frequency of 12.0±4.4 Hz. In contrast, during locomotion there was an increase in the frequency and amplitude of the junction potentials, followed by the discharge of several different fibres (Fig. 6A).

To explore whether the long latencies of attraction and withdrawal responses are due to the slow speed of movement of the animals or to a slow integrative mechanism in the nervous system, the latency of the response of the MCA was recorded and compared with the latencies of attraction and withdrawal responses in the actographic chambers. At 0.17 lx, the latency of the MCA was 1.18±0.42 min (N=14) and it increased to 3.66±0.34 min at 5.5 lx (N=12). These latencies were the same as those of the attraction response in the actographic chambers. In contrast, in the light intensity range that produced the withdrawal response, some differences were observed. At 5.6 lx the latency of the response of the MCA was 3.66±0.34 min (N=13) whereas the latency of withdrawal was 5.63±1.2 min (N=19). These differences were maintained with increasing light intensity until a level of 300 lx was reached. This produced a latency 0.21±0.16 min (N=10) in the MCA, whereas the latency for withdrawal was 1.79±0.63 min (N=18).

Illumination of the tail did not stimulate MCA activity below 88 lx. Above 88 lx, MCA response had a latency of 0.5±0.19 min, which was similar to the latencies of controls.

Discussion

Attraction and withdrawal responses are important adaptive mechanisms in crustaceans. Low light intensities attract crayfish out of burrows. This may be considered as an initial response which later gives rise to more sophisticated behaviour patterns such as searching for food, reproduction and other social interactions. Higher levels of light intensity give the animals a signal to withdraw, a response that protects them from predators.

Attraction and withdrawal responses were reproduced under laboratory conditions. Both are highly stereotyped behaviour patterns similar to those produced after stimulation of certain command neurones in the circumoesophageal connec-

tives (Bowerman and Larimer, 1974). The illumination range producing attraction covers about 1 log unit; over this light intensity range, a withdrawal response occurs. Eight low-threshold SFs were found to respond to light within the intensity range of the attraction response. In contrast, withdrawal seems to be mediated by a set of SFs with high thresholds and by the CPRs.

The threshold of the SFs is determined by the angle at which the eye receives light (Glantz, 1973). Owing to the spherical shape of the eye, high-threshold SFs do not receive light in a straight line when crayfish are inside the burrow. Thus, diffuse illumination induces an artificial separation of the thresholds of the SFs, giving rise to the two different groups described here. Collateral inhibition seems to play a role in this phenomenon (Wiersma and Yamaguchi, 1966; Aréchiga and Yaganisawa, 1973; Waldrop and Glantz, 1985), although the mechanism is not yet clear.

In the cerebral ganglion, SFs establish largely polysynaptic connections with descending interneurones that send their axons through the circumoesophageal connectives (Wood and Glantz, 1980*a*). Whether the targets of the SFs are command neurones for walking has not been established. It has been found that the bursting responses of the SFs improve temporal summation in visual descending neurones of the circumoesophageal connectives, augmenting synaptic efficacy (Wood and Glantz, 1980*b*). As a consequence, the desynchronisation of the bursting pattern of the SFs could participate in the switching between attraction and withdrawal by modifying the synaptic efficacy between SFs and descending interneurones.

The increase in the latency of attraction when the intensity of light increases is probably due to inhibition of the circuit for forward walking. This inhibition does not seem to occur at the level of the SFs, because no deflections in the slope of their dynamic range have been observed at high intensities. Moreover, the release of the attraction response seems to be linked to light adaptation of one screening pigment of the eye, which eventually decreases the amount of light that arrives at the photoreceptor cells, reducing the response of the SFs and switching off inhibition of forward walking (F. Fernández-de-Miguel and H. Aréchiga, in preparation). In contrast, the decrease in withdrawal latency with increasing light intensity could reflect an increase in the firing frequency of the sensory inputs.

In crayfish where the visual fields of the high-threshold SFs were painted, attraction behaviour persisted, but withdrawal was suppressed below 11 lx. This supports the idea that attraction is initiated by the low-threshold SFs whereas withdrawal has two sensory inputs. The experiments illuminating the retina or the tail of animals tested on the treadmill reinforced this idea, since forward walking can be obtained only when the retina is illuminated in the intensity range that causes attraction, whereas at higher intensities, illumination of the retina can only produce a slow withdrawal response. Moreover, to obtain a fast withdrawal response, the CPR must be illuminated.

It has been shown that intracellular stimulation of the CPRs is able to induce the excitation of command neurones for walking (Simon and Edwards, 1990). In

addition, when the sixth ganglion is illuminated, the responses of motoneurones are similar to those produced by stimulation of the command neurones for backward walking (Kovak, 1974). Nevertheless, the latency for backward walking is slower in animals with covered retinas (Edwards, 1984), supporting the idea that retinal and extraretinal photoreceptors participate in the withdrawal response.

Use has already been made of a treadmill device to study locomotion in lobsters (Ayers and Davies, 1977). In our studies, the use of a treadmill allowed us to analyse the relationship between light intensity and the direction of crayfish walking. Forward walking was observed over the range of light intensities that induced attraction, whereas backward walking was observed over the range of light intensities that induced withdrawal.

Ayers and Davies (1977) have shown that the MCA of the third walking leg participates in both forward and backward walking. This makes it possible that the latency for the response of this articulation reflects attraction and withdrawal responses. The shape of the curve relating latency to light intensity for attraction and withdrawal can be reproduced by recording the responses to light in the muscles of the walking legs. Over the intensity range for attraction, the latency for the response in the MCA increased, while over the intensity range for withdrawal the latency decreased. The MCA response and forward walking had latencies similar to that for attraction, while over the range of intensities for the withdrawal response, the latencies for the response of the MCA and for backward walking were significantly smaller than that for withdrawal. The differences between the latencies for withdrawal and MCA responses at the same light intensities could be explained by taking into account that the latency of the MCA response on the treadmill reflects only the latency of the endogenous integration mechanism, whereas the latency of the withdrawal response includes the time for the crayfish to find their burrows before the response is recorded.

The long latencies observed for attraction need an explanation. The latency of the response in the SFs to illumination was found to be between 20 and 120 ms, depending on the intensity applied; Glantz (1972) has found similar values. The latency for walking when the command neurones are stimulated at 50 Hz is 2 s (Bowerman and Larimer, 1974) and the latency of the response of the MCA to 0.17 lx was 1.18±0.42 min. Since the sum of the latencies of the responses of the individual elements of the circuit is much smaller than the behavioural latency, it is necessary to postulate the presence of a 'storage circuit' in the crayfish nervous system which keeps the information from the sensory inputs and releases the response after a certain delay. Since stimulation of the command neurones induces walking after 2 s, a probable anatomical location of the storage circuit is the cerebral ganglion. F. Fernández-de-Miguel and H. Aréchiga (unpublished observations) have shown that short pulses of light (30 s) can produce attraction responses with normal latencies, supporting the hypothesis of a storage mechanism.

F.F.-d.-M. was supported by CONACYT Fellowship 44365 and a complemen-

tary CINVESTAV Fellowship. We are greatly indebted to the Grass Foundation, which provided the P-15 preamplifiers used in the extracellular recordings. We also wish to thank Eduardo Nieto, Alejandro Garcia Moreno and Felipe Pacheco for participating in the design and construction of the actographic chambers and treadmill devices, and Victor Anaya for technical assistance. We also thank Drs John G. Nicholls, Robin Cooper and Mark Treherne for their critical comments on the manuscript.

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