12 Wiltschko, R., and Wiltschko, W., J. comp. Physiol. A *157* (1985) 469.
13 Wiltschko, W., Wiltschko, R., and Walcott, C., Behav. Ecol. Sociobiol. *21* (1987) 333.
14 Ganzhorn, J. U., Anim. Behav. *40* (1990) 65.
15 Wiltschko, W., Z. Tierpsychol. *25* (1968) 537.
16 Wallraff, H. G., Z. Tierpsychol. *30* (1972) 374.
17 Pianca, E. R., Evolutionary Ecology. Harper & Row, New York 1988.
18 Papi, F., Ioalé, P., Fiaschi, V., Benvenuti, S., and Baldaccini, N. E., Monit. zool. ital. (N.S.) *18* (1984) 53.

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Behavioral selection in crayfish correlates with movement of a screening pigment in the eye

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Abstract. Light induces two contrasting behavioral responses in crayfish: attraction at low intensities and withdrawal at high intensities. The aim of our experiments has been to study whether screening pigments of the eye influence the selection of attraction or withdrawal responses. During illumination, screening pigments mask photoreceptor cells, reducing the gain of the visual system. Comparison of the time and light-intensity functions of pigment migration and of attraction and withdrawal responses suggest that pigment migration might influence the selection as well as the latency of the response.
Key words. Behavior; crayfish; vision; pigment; invertebrate; Crustacea.

The nervous system responds to sensory stimulation with different behavioral patterns. At present, not much is known about how mechanisms for selecting behavioral responses are structured. Crayfish are useful for this type of study, because of their wide behavioral repertoire and the great amount of information available about the physiology of their nervous system.

Field observations and behavioral recordings under laboratory conditions have shown that different light levels induce contrasting patterns of burrowing behavior in crayfish and in other crustacean species: low intensities induce an attraction towards light, whereas higher intensities produce a withdrawal response[1,2]. The attraction response is restricted to a range of 1 logarithmic$_{10}$ unit of light intensities. Within this range, the latency of attraction (the time for the animal to emerge from the burrow after light onset) increases as a function of light intensity. A transition point exists where the attraction response is suppressed and a withdrawal reaction is produced. Withdrawal bears an opposite relation to light intensity: the latency (time for the animal to retreat to the burrow after light onset) becomes shorter as the intensity increases[1].

Behavioral and electrophysiological experiments suggest that attraction and withdrawal responses might be mediated by subsets of light-responsive sustaining neurons in the optic nerve[3] with low and high thresholds respectively[1]. Attraction is associated with the response of low-threshold sustaining fibers, whereas withdrawal is associated with the response of high-threshold sustaining fibers. In addition, the photoreceptor cells in the 6th abdominal ganglion[4] supply input to the withdrawal response[1,5].

At increasing light intensities, the rise in the latency of attraction correlates with the activation of high-threshold sustaining fibers. Therefore, the increase in the latency might be due to conflict over the selection of behavioral response, owing to the simultaneous activation of both inputs.

One possible mechanism underlying this complex behavioral integration might be the migration of the retinal screening pigments of the eye[6]. The migration of two different screening pigments modulates the amount of light arriving at the photoreceptor cells. Proximal pigment migration occurs as a response to background illumination. Light intensity determines the final position of proximal pigment without having any effect on the time course of migration[7,8]. By contrast, distal pigment migration requires the presence of the Distal Pigment Light-adapting Hormone[9]. Each pigment is known to modulate photon flux to the retinal receptors over a range of 1 logarithmic$_{10}$ unit[9].

The present report concerns the relationship between light-intensity, the latency of the behavioral responses, and the migration of screening pigments.

Methods

In all experiments, adult crayfish *Procambarus clarkii* of either sex were used at intermolt. Crayfish were kept in individual chambers with a simulated burrow under controlled light : dark (12 : 12) cycles. During the light period, a white light of 1200 lx was applied.

Behavioral experiments. The design of the chambers and the methods used to record attraction and withdrawal responses have been described elsewhere[1,10]. In brief,

each crayfish was kept in a two-compartment chamber at constant temperature (20 °C). One of the compartments simulated a burrow, whereas the other was a wider chamber. Locomotion of crayfish and their compartment changes were recorded by means of an opto-electronic system coupled to a computerized device[10]. Prior to the tests, crayfish were adapted to complete darkness for 90 min. Behavioral responses to light were studied during the first 3 h of the dark period, when crayfish show their maximum levels of spontaneous locomotor activity[11].

An attraction response was defined as the emergence of the crayfish from the burrow when a light was applied to the outer chamber. Withdrawal meant that the animal, previously in the open space, walked back to the burrow in response to illumination. Since in darkness crayfish walked spontaneously from one compartment to the other, the intervals spent by crayfish in each compartment in complete darkness were also measured. These values were used as references, so that after light onset, an attraction latency had to be smaller than the interval spent in the burrow in darkness, whereas a withdrawal latency had to be shorter than the interval spent spontaneously outside the burrow. Crayfish were readapted to dark before each subsequent trial. Data were expressed as the latency versus logarithm$_{10}$ of light intensity (log I) relationship.

Determination of screening pigment positions. The positions of screening pigments were determined by two different protocols: a) to measure the positions of screening pigments as a function of light intensity, groups of 4 crayfish were illuminated for 30 min, each with a different intensity; b) to measure the time course of the migration of screening pigments, groups of 4 animals were illuminated at 2000 lx and the positions of screening pigments determined at different times. All the experiments were carried out in the two-compartment chambers. When illumination was at intensities that produced an attraction response, the mouth of the burrow remained open. By contrast, when illumination was in the range of light intensities that produced a withdrawal response, the mouth of the burrow was closed to keep the animals in the wide chamber.

After illumination, crayfish were killed and pigment migration stopped by immersing the eyes in water at 90 °C for 1 min, in complete darkness[12]. Both eyes of each animal were transferred to a solution of 10% formaldehyde, and 24 h later the eyes were bisected and screening pigment positions were defined as indices by the formulae proposed by deBruin and Crisp[12] (fig. 1):

$$PPI = a/b \quad \text{and} \quad DPI = (d1 + d2)/2d3$$

where PPI is the proximal pigment index; 'a' is the distance from the basal membrane to the distal extreme of the proximal pigment; 'b' is the distance from the basal membrane to the distal extreme of the retinular cells; DPI, distal pigment index; d1, distance from the distal extreme of the distal pigment to the corneal surface; d2, distance from the proximal extreme of the distal pigment to the corneal surface; d3, distance from the corneal surface to the basal membrane (fig. 1). Data were expressed as mean values ± SE.

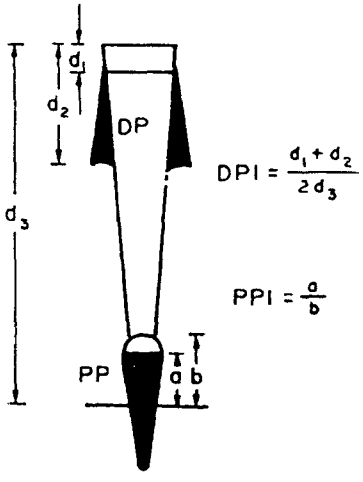

Figure 1. Criteria for defining the position of the screening pigments. PP, proximal pigment; PPI, proximal pigment index; a, distance from the basal membrane to the distal extreme of the proximal pigment; b, distance from the basal membrane to the distal extreme of the retinular cells; DP, distal pigment; DPI, distal pigment index; d1, distance between the distal extreme of the distal pigment and the corneal surface; d2, distance from the proximal extreme of the distal pigment to the corneal surface; d3, distance from the corneal surface to the basal membrane. Adapted from deBruin and Crisp[12].

Positions of both pigments were measured in the central region of the eye, since this area contains the sensory fields of the sustaining fibers participating in the attraction response.

Results

Behavioral experiments. Attraction and withdrawal responses to light were studied in 9 animals. An attraction response was observed when a light with an intensity between 0.17 and 1.4 lx was turned on outside the burrow[1]. The shortest latency (1.82 ± 0.6 min) was observed at 0.17 lx. Lower light intensities did not produce any response. Increasing light intensities were associated with increases in the latency of attraction (fig. 2). At 2.8 lx, the latency of attraction was 5.08 ± 0.4 min, similar to the spontaneous interval spent inside the burrow in darkness (6.22 ± 0.45 min). The increase in the latency of attraction from 0.17 lx to 2.8 lx was 4.26 min. Transition between attraction and withdrawal responses occurred at 2.8 lx (fig. 2).

A withdrawal response occurred with light levels above 2.8 lx. At 2.8 lx, the withdrawal latency was 7.49 ± 1.1 min, equal to the interval that crayfish spent spontaneously outside the burrow (7.11 ± 0.94 min). The withdrawal latency decreased with increasing light intensities; for example, at 1050 lx, the latency was 1.7 ± 0.8 min (fig. 2).

Response to light by the screening pigments. To compare the light intensity relationships of the behavioral re-

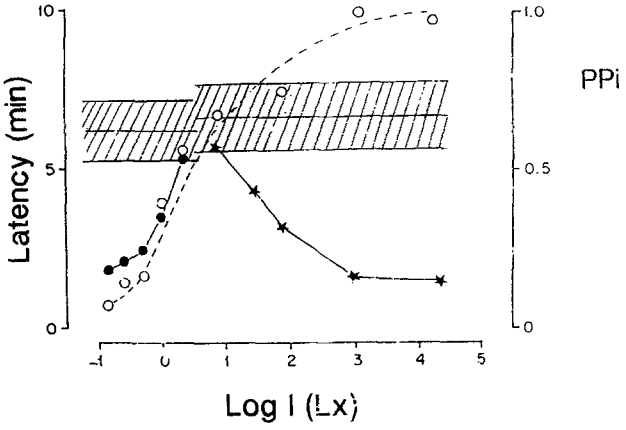

Figure 2. Attraction (black circles) and withdrawal (black stars) latencies are compared with PPI values (white circles) at different light intensities. The mean value ± SE of spontaneous emergence and return to the burrow in darkness are represented by the parallel lines above attraction and withdrawal latencies, respectively.

Light intensity (lx)	AL (min)	PPI	PPM (min)	AL-PPM (min)
0.17	1.82	0.07	0.00	1.82
0.34	2.18	0.14	0.50	1.68
0.70	2.28	0.16	0.56	1.72
1.40	3.63	0.39	2.28	1.35
2.80	5.08	0.56	3.50	1.58

The three columns in the middle show the experimental mean values of attraction latencies (AL), proximal pigment index (PPI) and the calculated duration of proximal pigment movement (PPM) respectively, related to the light intensity (left column). The right hand column shows the values obtained after subtracting the duration of proximal pigment movement from the attraction latencies at different light intensities (AL-PPM). Mean values of 8 observations are presented; SE given in the text. PPI values were obtained from equation 2.

sponses and screening pigment migration, the positions of screening pigments were determined at different light intensities. Each group of 4 dark-adapted crayfish was illuminated for 30 min at a different intensity. In control animals in complete darkness, the proximal pigment was found close to the basal membrane, in a dark-adapted position (PPI = 0.06 ± 0.04). The threshold for proximal pigment migration (defined as the lowest light intensity tested at which PPI > 0.1) was found at 0.34 lx (PPI = 0.14 ±0.09). Proximal pigment migrated to more light-adapted positions as the light intensity was increased (fig. 2), in agreement with previous observations[7,8,12]; for example, with 5.6 lx PPI = 0.68 ± 0.02. Full light adaptation was found with 1000 lx (PPI = 1.0). Figure 2 shows attraction and withdrawal latencies and proximal pigment positions at different light intensities. As can be seen, the threshold for proximal pigment migration as well as the first increase in the latency of attraction were found at 0.38 lx. At the light intensity corresponding to the transition between attraction and withdrawal responses, the proximal pigment had migrated 56% of the distance towards complete light adaptation.

Changes in the light levels produced only small changes in distal pigment position. The distal pigment was found partially light-adapted under complete darkness (DPI = 0.059 ± 0.006) and over the whole range of light intensity tested[9]; for example, at 88 lx, DPI = 0.062 ± 0.004. Above 88 lx, a slight but not significant migration towards a more light-adapted position was observed; for example, at 1000 lx, DPI = 0.072 ± 0.07.

Time course of screening pigment migration. It has been shown that the time course of light-induced proximal pigment migration is independent of the light intensity[7,8]. Therefore, the migration time of the proximal pigment was studied in groups of eight animals at different times of exposure to light at 2000 lx. Light adaptation of the proximal pigment occurred in two stages[7]. In the first stage, the proximal pigment moved from PPI = 0.06 ± 0.04 at time (T) = 0.0 min, to PPI = 0.68 ± 0.3 at T = 4 min (table I). A significant relationship (r2 = 0.999) between PPI and T was found for this stage (equation 1).

$$PPI = 0.14\ T + 0.07 \qquad \text{Eq. 1}$$

The second migration stage was slower. Full light adaptation of proximal pigment was reached at T = 12 min (PPI = 1.0). During the whole period, the position of distal pigment remained unchanged.

To correlate proximal pigment migration with the increase in the attraction latency at increasing light intensities, the duration of proximal pigment migration was calculated at different light intensities. Equation 1 was arranged to express PPI as an independent variable (equation 2) and PPI values at different light intensities (fig. 2) were substituted into equation 2 (table).

$$T = (PPI - 0.07)/0.14 \qquad \text{Eq. 2}$$

Data from equation 2 showed that the duration of proximal pigment migration was similar to the increase in attraction latency at every light intensity tested. To provide further support for this, the values obtained from equation 2 were subtracted from the latency of attraction at every light intensity (table). As can be seen in figure 3, the subtracted values at different light intensities resembled the latency of attraction at threshold.

Another correlation was found between the transition from attraction to withdrawal responses and the migration of the proximal pigment. At 2.8 lx, the increase in the latency of attraction was 3.1 min, whereas the calculated duration of proximal pigment migration was 3.5 min (table), corresponding to the first stage of proximal pigment migration. By contrast, the difference between the longest (7.49 ± 0.8 min, at 2.8 lx) and the shortest (1.7 ± 1.1 min, at 1050 lx) latencies of withdrawal was 4.38 min, corresponding to the beginning of the second stage of proximal pigment migration.

Discussion

The time and light intensity functions of proximal pigment migration and the attraction response share several

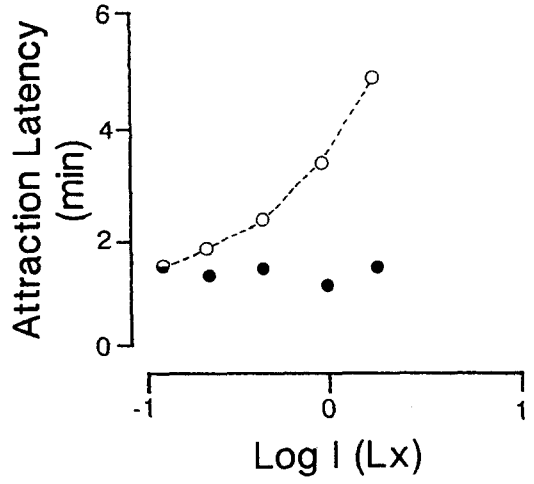

Figure 3. The latency for the migration of the proximal pigment to its steady state position at every intensity was subtracted from the latency of attraction (white circles). At all intensities the result of the subtraction gave values similar to the latency of attraction at threshold (black circles).

characteristics. The threshold for proximal pigment migration was found at 0.34 lx, corresponding to the first increase observed in the latency of attraction. From this point, the duration of proximal pigment migration equalled the increase in attraction latency at every light intensity tested. Interestingly, high-threshold sustaining fibers O14, O30, O9 and O38[3], participating in the withdrawal response have their thresholds at 0.09 lx, 0.17 lx, 0.38 lx and 0.38 lx respectively[1], close to the thresholds of the attraction response (0.17 lx) and proximal pigment migration (0.34 lx).

It is known that proximal pigment migration to a full light-adapted position reduces photon flux to the photoreceptor cells by 1 logarithmic unit[9]. Since light adaptation of the proximal pigment was only 56% between 0.38 and 2.8 lx, the reduction of photon flux to the photoreceptor cells cannot totally compensate for the increase in light intensity. Nevertheless, in previous experiments it was observed that sustaining fibers reach their dynamic ranges in less than 0.5 logarithmic units above their threshold[1]. Therefore, the migration of proximal pigment might be enough to reduce the firing frequency of high-threshold sustaining fibers to levels below their dynamic ranges, thus removing the conflict in behavioral selection.

At the light intensity where the transition from attraction to withdrawal has been observed, proximal pigment migration reaches the threshold of its second stage. The functional role of this effect in behavioral selection is difficult to predict, as the withdrawal latency decreases at higher illumination levels, correlating with increases in the firing responses of participating interneurones.

Under our experimental conditions there is probably no contribution of distal pigment to attraction and withdrawal responses, since no positional changes were observed in response to illumination. Nevertheless, it is known that the distal pigment buffers the amount of light to the photoreceptor cells by 1 logarithmic unit over the 24-h cycle[9]. Therefore, it is possible that distal pigment influences the thresholds for attraction and withdrawal responses over a 24-h period.

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1 Fernández-de-Miguel, F., and Aréchiga, H., J. exp. Biol. *164* (1992) 153.
2 Aréchiga, H., and Atkinson, R. J. A., Mar. Biol. *3* (1975) 63.
3 Wiersma, C. A. G., and Yamaguchi, T., J. comp. Neurol. *128* (1966) 333.
4 Kennedy, D., Ann. N.Y. Acad. Sci. *74* (1958) 329.
5 Simon, T., and Edwards, D. H., J. comp. Physiol. *A 166* (1990) 745.
6 Rao, K. R., in: The Biology of Crustacea, vol. 9, p. 395. Eds D. E. Bliss and L. H. Mantel. Academic Press, New York 1985.
7 Olivo, R. F., and Larsen, M. E., J. comp. Physiol. *A 125* (1978) 91.
8 Frixione, E., and Pérez-Olvera, O., J. Neurobiol. *22* (1991) 328.
9 Rodríguez-Sosa, L., and Aréchiga, H., Vision Res. *22* (1982) 1515.
10 Fernández-de-Miguel, F., Cohen, J., Zamora, L., and Aréchiga, H., Biol. Estud. Med. Biol. Mex. *3–4* (1989) 71.
11 Fernández-de-Miguel, F., and Aréchiga, H., Soc. Neurosci. Abstr. *14* (1988) 1299.
12 deBruin, G. H. P., and Crisp, D. J., J. exp. Biol. *34* (1957) 447.

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