Development of Visual Pattern Discrimination in the Fly Depends on Light Experience

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Pattern discrimination by dewinged walking flies (*Boettcherisca peregrina*) was tested in behavioral experiments. After emergence, the flies were deprived of light or visual patterns. Deprivation impaired the normal development of visual pattern discrimination without impairing phototaxis. Flies kept in a lighted, white, unpatterned environment could not discriminate visual patterns, nor could flies kept in continuous darkness. These results indicate that there is considerable plasticity in the structure of the visual system of these flies.

THE ADULT INSECT NERVOUS SYSTEM is usually considered to be a hard-wired network lacking structural plasticity, although modification of insect behavior produced by individual experience has been demonstrated (*1*). I investigated the influence of the deprivation of light or visual patterns on pattern discrimination as an example of higher visual function.

The approach used was based on neurophysiological and behavioral evidence for discrimination of several visual patterns by the fly (*2, 3*). When dewinged flies that were raised in a normal light-dark (LD) environment were put at one end of an arena, they walked toward an illuminated pattern target at the other end. This behavior was reinforced by the flies' positive phototaxis, but arrival at the target also depended on the pattern shapes, suggesting, in addition, pattern discrimination.

I used two kinds of patterns for testing flies that had been raised under different light conditions (Fig. 1). The patterns were selectively adopted from eight used in earlier studies (*3*), because one of them (a star shape) was most attractive for flies and the other (an oblique bar) was least attractive (Fig. 2E). The targets (diameter, 2.5 cm) were made of thin, white paper on a black background, were evenly transilluminated from behind, and had stripes of equal width (5 mm). Luminosity of the patterns was 1.2 cd (*4*).

In the first experiment, one of the two patterns was put at one end of an arena (35 by 35 cm). The black-painted floor of the arena was surrounded by black walls 5 cm high and was illuminated by a light on the ceiling. The illumination intensity of the arena was 6.0 lux (*4*). Flies were placed at the end opposite that of the target, toward which they walked freely. They had been raised at 25°C in the dark from the beginning of pupation, and had been light-deprived after emergence. They were exposed to a usual LD environment only after the day of the first trial for pattern discrimination. In this light environment, flies were kept in a transparent cage and so could see all of their surroundings. Flies were exposed to each target pattern 40 times. The ratio of successful arrival frequencies to all trial frequencies was calculated as the rate of arrival.

When flies were tested for discrimination immediately after emergence, they could not discriminate between the patterns and showed weak phototaxis. One day after emergence, phototaxis was augmented but pattern discrimination was still not apparent. After 2 days, however, the rate of arrival at the star-shaped pattern was strikingly increased and that to the oblique bar was decreased (Fig. 1A). Such enhancement of discrimination was also seen in flies kept in the dark for up to 4 days after emergence (Fig. 1, B to E). In addition, a period of 4 to 6 days after the beginning of exposure to a light environment was necessary for the

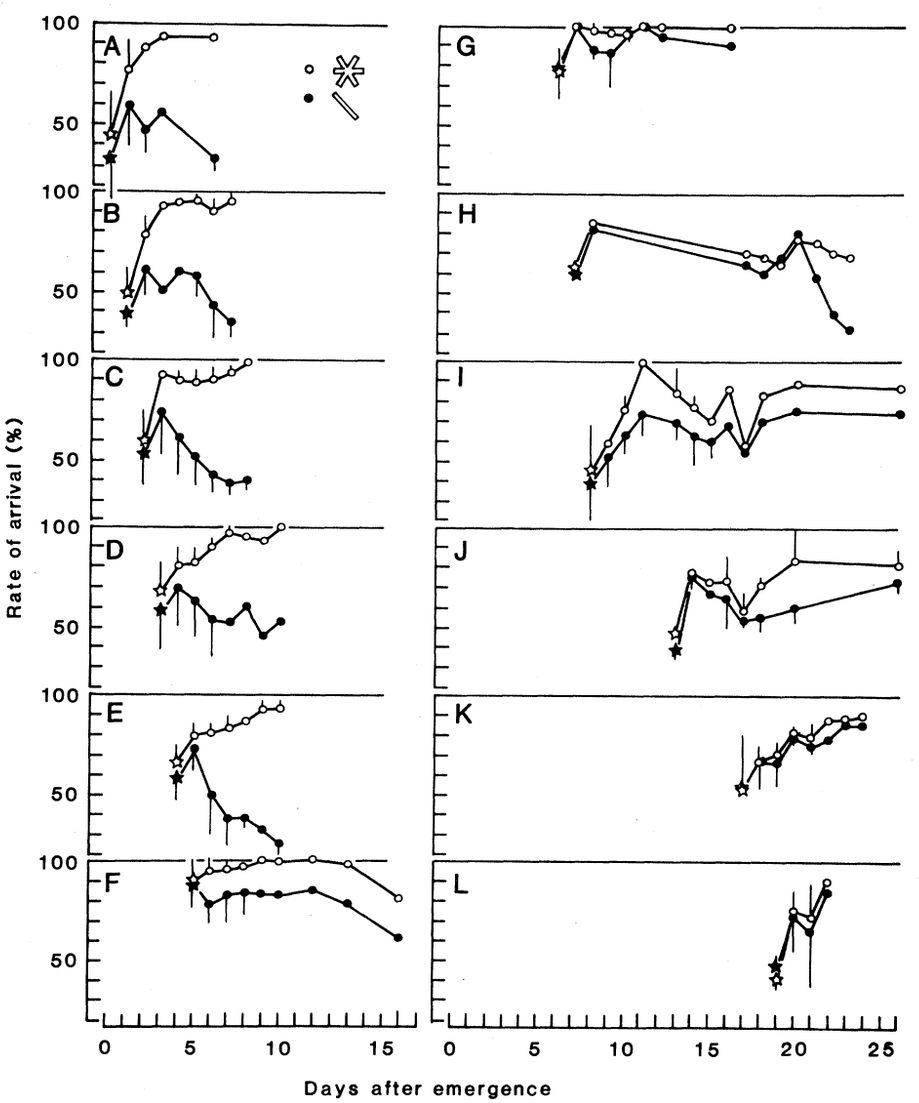

Fig. 1. Rate of arrival at the star-shaped pattern (○) and the oblique bar pattern (●) in walking flies that were raised in the dark for various periods after emergence. Days on which LD started are shown by special symbols (☆, ★). Error bars indicate standard deviations. The data points represent the average responses of three to six flies, except in (H) and a part of (F), (G), and (I) (no error bar points), which were obtained from the response of one fly.

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complete development of pattern discrimination.

This developmental tendency was not observed, however, in flies kept in the dark for more than 4 days after emergence (Fig. 1, F to L). In these flies, phototactic behavior was still enhanced, but discrimination between the two patterns failed to appear even though tests were repeated for 2 weeks or more (*5*). This shows that short-term light experience by day 4 after emergence is important for the development of visual pattern recognition and that light deprivation does not impair the sensitivity to light but only pattern discrimination.

In a second series of experiments, I investigated whether a particular pattern, delivered to flies for a short period after emergence, determines the property of pattern recognition. Flies that had been raised in continuous darkness from the beginning of pupation were put in a pattern-free apparatus immediately after emergence (Fig. 3). In this apparatus pieces of paper on which right-down oblique, horizontal, or vertical stripes were drawn were fixed on the outside of a petri dish 20 cm in diameter and 5 cm deep. Black and white stripes were about 2 to 3 mm wide. White paper was rolled on the outside to give a lighted but unpatterned environment. A second petri dish 9 cm in diameter was overturned and fixed at the center of the large petri dish. The apparatus was illuminated from above by two fluorescent lamps; illumination at the wall was 36 lux (*4*). Water was poured into the large petri dish to a level just below the top of the small petri dish. A dewinged fly was put on the top of the small petri dish, and was thus contained by a moat. By this procedure, flies always looked at surroundings having a particular display through most of their compound eyes (*6*). The flies experienced the display every 5 hours for 5 days, since, as shown above, 4 to 6 days were necessary for the complete development of pattern discrimination.

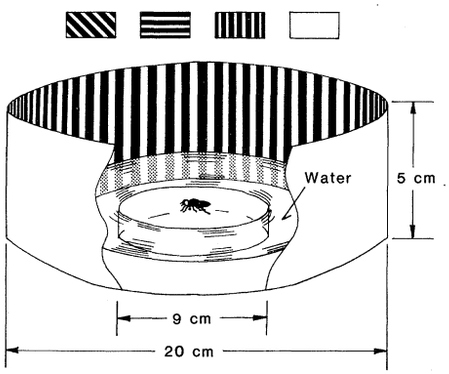

Fig. 3. Apparatus for deprivation of visual pattern. Samples of the displays are illustrated at the top.

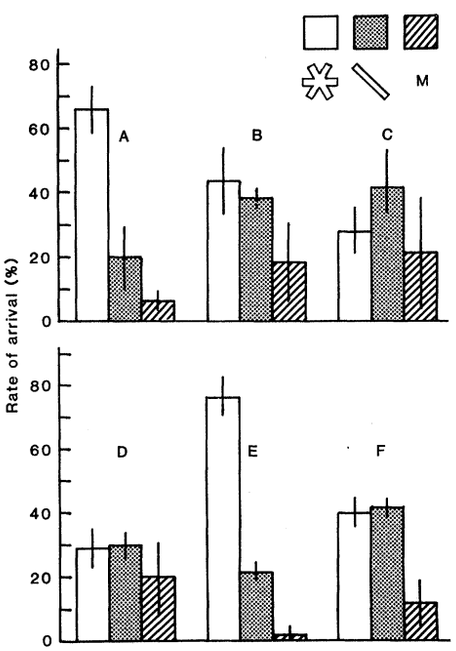

Fig. 2. Rate of arrival at each of paired patterns of the star shape and oblique bar (inset) in walking flies that had experienced the different displays. M represents arrival at the mid-point between two targets. Results are illustrated for flies kept in the horizontal (A), vertical (B), and right-down oblique stripe displays (C) and the white, unpatterned display (D) and for flies exposed to a normal, patterned environment with the same conditions as the pattern-deprived flies (E); (F) Results from flies raised in continuous darkness for 5 days after emergence. The data were the averaged values of three to five flies for each pattern deprivation. Error bars indicate standard deviations.

After exposure for 5 days, flies were tested for pattern discrimination every day from day 6 to day 9 after emergence (*7*). The tests were performed by observing choice behavior between two simultaneously presented visual patterns. Each pair of the several visual patterns shown in Figs. 2 and 4 was presented at one end of the arena. The two targets were 9 cm apart, and the positions of each of the two patterns were exchanged every ten times in all 20 trials to avoid a reaction to the position. Flies were placed at the end opposite to that of the pattern targets, toward one of which they walked freely. The size and illumination of the targets were the same as described for the first experiment.

Pattern discrimination was first tested for by choice behavior involving a star-shaped pattern and a right-down oblique bar symbol (inset in Fig. 2). Flies that had been exposed to the horizontal stripes predominantly chose the star-shaped pattern (Fig. 2A). This was similar to the results for flies raised under normal light conditions (Fig. 2E). Different features were seen, however, in flies exposed to the vertical and oblique stripes (Fig. 2, B and C); neither displayed strong attraction to the star-shaped pattern and the rates of arrival at both patterns were almost the same.

Flies that had experienced the normal, lighted environment could clearly discriminate between the two patterns (Fig. 2E). However, flies that had been kept in the white, unpatterned environment were unable to discriminate between the two patterns (Fig. 2D). The result was the same for flies kept in continuous darkness (Fig. 2F), although the arrival rates were actually slightly higher (*8*).

Further observations were made with respect to the choice behavior between two contrasting patterns (Fig. 4). In flies that had been exposed to horizontal stripes, the rate of arrival at a horizontal bar was very high compared to that for a vertical bar (Fig. 4A). On the other hand, flies that had been exposed to vertical stripes chose the vertical bar target rather than the horizontal one (Fig. 4B). Lastly, in flies that had been exposed to right-down oblique stripes, the rate of arrival at the right-down target was significantly higher than that for the left-down one (Fig. 4C).

In contrast to the situation with vertebrates (*9*), only a few electrophysiological and histological studies have been undertaken on sensory deprivation in invertebrates (*10, 11*), and the findings have been inconsistent. The behavioral experiments presented here demonstrate that, even in the fly, normal development of visual pattern discrimination requires not only a lighted environment but also the presence of a visual

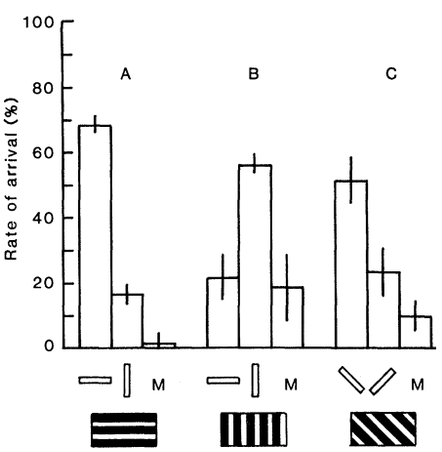

Fig. 4. Rate of arrival at each of paired visual patterns, one of which had the same property as the display and the other of which had the contrasting property. Samples of the displays and patterns presented are shown at the bottom. The data are the averaged values for three to five flies for each pattern deprivation. Error bars indicate standard deviations.

environment with a variety of patterns for a short period after emergence (*12*). Since there are striking similarities between the phenomena observed in the fly and those observed in vertebrates, it seems that there are also similarities in the neuronal and molecular bases of the modification of the developing nervous system. One possibility is a change in synapses through disuse (*11*).

REFERENCES AND NOTES

1. R. Menzel and J. Erber, *Sci. Am.* **239**, 102 (July 1978).
2. K. Mimura, *J. Comp. Physiol.* **141**, 349 (1981).
3. _____, *ibid.* **144**, 75 (1981); *ibid.* **146**, 229 (1982).
4. Luminosity was measured by putting a photosensitive unit of a conventional photometer at the patterns and illumination intensity was measured by putting the photosensitive unit on the arena or the wall.
5. A small preference for the star target was seen, but was clearly of much smaller magnitude than that shown in Fig. 1, A to E.
6. It may be irrelevant that the dorsoanterior eye regions were not covered by the display, because this region of the eye was not used to test for pattern discrimination.
7. An important problem is the stability or persistence of deprivation effects. Recovery from deprivation effects seems to depend on the length of deprivation and on the conditions of the light environment experienced after deprivation.
8. The rates of arrival in Fig. 2F are lower overall than in those of Fig. 1F, in spite of similar experimental conditions. Reasons for the difference are not evident. But, considering the difference in experimental conditions between both experiments, a possible reason may be a difference in the temperature of the experimental room. The experiments represented in Fig. 1 were performed at 25° to 26°C (in autumn), while those in Fig. 2 were done at 20° to 22°C (in winter). Lowering of temperature may change the flies' general activity. The other possible reason is an effect of diurnal activity rhythms. Experiments represented in Fig. 1 were usually carried out in the evening (4 to 7 p.m.), while those in Fig. 2 were done in the afternoon (1 to 3 p.m.). However, a difference in the rates of arrival (discrimination) is important in the results presented here. The discrimination represented in Fig. 1F was small and was completely different from the tendencies illustrated in Fig. 1, A to E. So, it is reasonable to conclude that discrimination is scarcely possible under these conditions.
9. K. L. Chow, in *Handbook of Sensory Physiology*, R. Jung, Ed. (Springer-Verlag, New York, 1973), vol. 7, pp. 599–627; B. Skarf, *Brain Res.* **51**, 352 (1973); R. W. Guillery, *J. Comp. Neurol.* **149**, 423 (1973); H. V. B. Hirsch and D. N. Spinelli, *Science* **168**, 869 (1970); C. Blakemore and G. F. Cooper, *Nature (London)* **228**, 477 (1970); W. Singer, in *The Biology of Learning*, P. Marler and H. S. Terrace, Eds. (Springer-Verlag, New York, 1984), pp. 461–477.
10. J. W. Bloom and H. L. Atwood, *J. Comp. Physiol.* **135**, 191 (1980); S. G. Matsumoto and R. K. Murphey, *J. Physiol. (London)* **268**, 533 (1977); G. M. Technau, *J. Neurogenet.* **1**, 113 (1984); K. Hausen, in *Photoreception and Vision in Invertebrates*, M. A. Ali, Ed. (Plenum, New York, 1984), pp. 523–559; H. Hertel, *J. Comp. Physiol.* **147**, 365 (1982).
11. H. Hertel, *J. Comp. Physiol.* **151**, 477 (1983).
12. According to results obtained from many flies in experiments in progress, a sexual difference was not observed.

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Differential Conditioning of Associative Synaptic Enhancement in Hippocampal Brain Slices

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An electrophysiological stimulation paradigm similar to one that produces Pavlovian conditioning was applied to synaptic inputs to pyramidal neurons of hippocampal brain slices. Persistent synaptic enhancement was induced in one of two weak synaptic inputs by pairing high-frequency electrical stimulation of the weak input with stimulation of a third, stronger input to the same region. Forward (temporally overlapping) but not backward (temporally separate) pairings caused this enhancement. Thus hippocampal synapses in vitro can undergo the conditional and selective type of associative modification that could provide the substrate for some of the mnemonic functions in which the hippocampus is thought to participate.

THE HIPPOCAMPUS IS A CORTICAL structure that has been strongly implicated in certain mnemonic functions (*1*). Some of the information processing that occurs in this region has been described in terms of a general spatiotemporal theory of higher-order Pavlovian conditioning (*2*). Hippocampal synapses can show rapid and persistent (*3*) associative changes when subjected to brief bursts of high-frequency electrical stimulation (*4*). Here we use a pattern of stimulation that shares formal features with differential Pavlovian conditioning to begin to elucidate these changes in the hippocampal brain slice.

Rat hippocampal slices were prepared and maintained in the conventional manner (*4, 5*). Three stimulating electrodes were placed in the Schaffer collateral and commissural projection to region CA1 (Fig. 1A) (*6*). The current delivered to one stimulating electrode [strong (S)] was set to elicit an extracellular synaptic response of approximately 2.5 mV [the strong (S) response]. The current delivered to the other two electrodes [weak 1 (W1) and weak 2 (W2)] was set to give much weaker synaptic responses—between 200 and 300 μV [the weak (W) responses]. Typical W and S synaptic responses are illustrated elsewhere (*4*). All responses were measured with a single extracellular electrode placed in the dendritic region between W1 and W2 (Fig. 1A).

Each weak synaptic input was tested once every 12 seconds, with W2 following W1 by 6 seconds. The continuous testing was punctuated by several types of conditioning trains (Fig. 1B). First, five conditioning trains (100 Hz for 600 msec) were applied to W1 and W2 to verify that such activity alone fails to induce long-term potentiation (LTP) in either of these two W responses (*4*). The interval between the onsets of the stimulation trains delivered to W1 and W2 was 800 msec, and the intertrial interval between each of the five W1-W2 pairings was 6 seconds (Fig. 1B). Second, five conditioning trains (100 Hz for 400 msec) were also delivered to S to verify that such activity alone does not produce heterosynaptic LTP in either of the W synaptic responses (*7*). Third, five conditioning trains were delivered to all three synaptic inputs with either the W1-S or the W2-S forward-pairing scheme (Fig. 1B). During W1-S forward-pairing the W1 trains began 200 msec before the S conditioning trains (forward pairing) and the W2 trains began 600 msec after the S trains (backward pairing); in the W2-S forward-pairing situation, these temporal relationships were reversed (*8*). To assess the effects of W-S pairings, we determined the W1 and W2 amplitudes by calculating the mean of ten consecutive responses obtained during a 2-minute period before and again after W-S pairing. The first 2-minute average was obtained immediately prior to W-S pairing. The second 2-minute average was taken between 12 and 16 minutes after W-S pairing.

Table 1. Synaptic response amplitudes as a function of forward (W+) and backward (W−) pairing. All values expressed as mean ± SEM.

Response	Amplitude		Mean increase (%)
	Before pairing (μV)	After pairing (μV)	
W+	230 ± 18	320 ± 19	42 ± 4
W−	244 ± 17	254 ± 20	5 ± 2

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