

# Conditioned Visual Flight Orientation in *Drosophila melanogaster* Abolished by Benzaldehyde

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LIU, L., X. WANG, S. XIA, C. FENG AND A. GUO. *Conditioned visual flight orientation in Drosophila melanogaster abolished by benzaldehyde*. PHARMACOL BIOCHEM BEHAV 61(4) 349–355, 1998.—To study learning and memory of *Drosophila melanogaster* in the flight simulator, single flies are trained in an operant conditioning paradigm to avoid a course towards visual objects that are associated with heat. The results demonstrate that normal flies (wild-type “Berlin”) can quickly learn to avoid the heat-associated objects and retain the memory. This allows us to further explore a recently communicated long-term effect of preimaginal benzaldehyde (BAL) influence on the association of visual objects with the aversive odor of BAL. Here we show that flies, exposed to BAL in the embryonic, larval or adult stage, do not accompany by a similar indifference to heat shocks. The flies are successfully trained to avoid the heat-associated visual patterns. It shows that the learning acquisition is not affected during the operant conditioning in the flight simulator. Neither object perception nor object discrimination is altered after exposure to BAL, as is shown by Fourier analysis. However, the test results after training show that exposure to BAL interferes directly with memory formation in *Drosophila*. It takes at least two generations of growth on the noncontaminated medium that the strain recovers fully from BAL-induced amnesia. The BAL treatment seems not only to affect the associative memory formation, but in addition the flies’ development in general, as is indicated by prolonged developmental time. However, the impaired memory after BAL treatment in different stages seems to have nothing to do with an influence of BAL on the cAMP-level. © 1998 Elsevier Science Inc.

*Drosophila melanogaster*    Benzaldehyde    Visual associative learning    Operant conditioning  
 Flight orientation    cAMP

FRUIT flies can learn a variety of nonassociative and associative tasks (16,24,31). Among associative tasks, classical olfactory associative learning and memory has extensively been investigated (26,33,35). The model of the genetic pathway involved with memory formation after classical olfactory conditioning shows that the information acquired during learning is processed into consolidated memories by passing earlier memory (8,35). Based on the flight orientation behavior, a learning paradigm has been introduced in the flight simulator for *Drosophila* (17,18,36–38). In this learning paradigm, the flight simulator is turned into an operant conditioning paradigm in which the fly learns to control the appearance of a reinforcement (heat) by its choice of flight direction with respect to the angular positions of visual patterns at the arena wall. In recent years, it has been shown that learning and memory of flies depend on several variables such as age, prac-

tice, and diet, that are significant for the performance in this learning paradigm (13,40). In agreement with the work on olfactory associative learning in *Drosophila*, behavioral and pharmacological analysis has provided clear evidence for at least four distinct phases in memory formation of flies after operant conditioning (41,42).

This operant conditioning offers a crossmodal association of visual cues with either heat or olfactory cues in *Drosophila*. It may help to investigate the confluence of different sensory modalities in the insect nervous system. Using the repellent odor benzaldehyde (BAL) as negative reinforcement, *Drosophila* was trained to avoid to flight towards the direction of one of two objects that had been associated with benzaldehyde. Early exposure to this substance in the larval stage, and presumably even in the embryonic stage, increased the indifference towards this odor in the adult flies (14). This indiffer-

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ence may either be interpreted as memory deficiency, or as a consequence of an adaptation of the flies to BAL. However, if heat is used as negative reinforcer instead of BAL, adaptational effects can be excluded.

The aim of the present work is: (a) to study on learning and memory of flies that had been exposed to BAL, using visual patterns as cues and heat as negative reinforcement. Such an experiment would indicate whether BAL disturbs the associative learning and memory between visual patterns and heat; (b) to measure the amount of cAMP in the heads of experimental flies which are exposed to BAL.

## METHOD

### Flies

*Drosophila melanogaster* of the wild-type strain "Berlin" was obtained from the Max-Planck Institute of Biological Cybernetics, Tuebingen. Flies were grown at  $24 \pm 1^\circ\text{C}$  in a 14 L:10 D cycle with light on at 0700 h, and normally bred on standard corn meal/molasses food medium ["Wuerzburg recipe" for details, see (13,40)]. Three- to 4-day-old flies were used in all experiments.

### Olfactory Adaptation

For BAL-treated flies, 0.05g BAL was added to 1 liter of food medium (14). Test flies were randomly selected from four simultaneously raised groups: (a) the control group (C-group): flies without any previous exposure to BAL; (b) the "embryonic-exposure" group (E-group): these flies received BAL by maternal exposure only in their embryonic stage. To obtain these flies, freshly hatched virgin females of the C-group were transferred to a tube containing about ~60 ml BAL-contaminated medium. After 1 day, males of the C-group were added to this tube. Two days after their inset, the fertilized females were transferred on noncontaminated medium in an interim tube, where they were likely to lose most of the incorporated BAL by excretion, diffusion, and chemical transformation. From day 4 to day 5, the flies were allowed to lay eggs on the noncontaminated medium in another tube. The emerging E-group of flies were collected from this tube. We also reared F1 and F2 generations of the E-group flies; (c) the "larval-exposure" group (L-group): these flies received BAL only in their larval stage. They were obtained from eggs laid by fertilized females of the C-group during temporary transfer to a tube containing BAL-contaminated medium. From hatching to pupation, the larvae fed on this medium. During this periods of about 10 days, the contamination was repeatedly refreshed because of its gradual oxidation to benzoic acid. The emerging L-group flies were collected in a tube containing noncontaminated medium; (d) the "adult-exposure" group (A-group): these flies received BAL in their adult stage. To obtain these flies, freshly hatched flies of the C-group were transferred to a tube containing BAL-contaminated medium for 1 day, then the flies were transferred to the noncontaminated medium. The A-group flies were collected from this tube.

### cAMP Level Tests

Cyclic AMP was measured in a radioimmunoassay where *Drosophila* cyclic AMP competed with a standard amount of tritium-labeled cyclic AMP for antibody binding sites. The method according to Davis et al. (5) was slightly modified: The heads of 3- to 4-day-old flies were homogenized in HCl at room temperature. The homogenates were centrifuged at

$25,000 \times g$  for 15 min, the supernatant was neutralized to pH 7.0, and centrifuged again at  $25,000 \times g$  for 15 min. The extracts were dried in a  $70^\circ\text{C}$  water bath, and diluted with Tris-HCl buffer, pH 7.5. The recovery of cAMP was estimated as described elsewhere (2,5).

### Flight Simulator

The flight simulator has been described before (13,17,18, 36,38,40). Briefly, the flight simulator establishes normal negative feedback between the fly's yaw torque and the angular velocity of a visual panorama surrounding the animal [closed loop; coupling coefficient  $K = -11^\circ(\text{s} \cdot 10^{-10} \text{ Nm})^{-1}$ ; for details, see (18,38)]. In this setup, the test fly stabilizes the angular velocity of the panorama and chooses its direction of flight with respect to the visual panorama consisting of two upright and two inverted T-shaped patterns (total horizontal and vertical width of figures  $40^\circ$ , of bars  $8^\circ$ ), which are displayed in alternating sequence in the four quadrants of a brightly illuminated arena (background luminance  $I = 400 \text{ cd/cm}^2$ ). Negative reinforcement is provided by the heat beam of a microscope lamp (Zeiss, 6 V, 15 W), which is switched on during training, but not during the test. The beam is focused from behind and above onto the fly by a lens ( $f = 60 \text{ mm}$ ), and can be intercepted by a computer-controlled shutter. The heat spot with an intensity of about 43 mW is about 3 mm in diameter.

For flight in the flight simulator, a small hook of copper wire (0.05 mm diameter) was glued to head and thorax of a 3-, 4-day-old test fly (13,18). Then the fly was put into a small transparent chamber for 1 night to adapt to its new situation. The following day the experiment was carried out. No sex-related differences in the learning ability were apparent. Therefore, male and female flies were used in all experiments.

### Standard Conditioning Procedure

The conditioning procedure was basically the same as in previous experiments (13,39,40,42). In brief, training and testing were performed in the following order: the pretraining session, two training sessions, and the test session. The pretraining session comprises three consecutive 2-min test periods during which the flying fly learns how to stabilize the panorama in closed loop without heat reinforcement (13,18,38). Simultaneously, its spontaneous preference with respect to the shape of visual patterns was tested. The training session comprises two 2-min training periods and one 2-min test period. During the training periods, the computer-controlled infrared beam, focused onto the fly, was switched on whenever the fly was flying towards a quadrant that contained, for instance, an upright T. When one of the both the inverted Ts was in the frontal visual field, the beam was intercepted by the computer-controlled shutter. In the following 2-min test period, the fly was tested for its learning acquisition. Then the training session was repeated. Finally, the fly was tested for memory formation in a test session of three 2-min test periods without heat reinforcement. Before each 2-min period, the panorama was set to a new random position. The total time of training and testing in single fly is about 24 min. Half of the flies in one group were trained and tested as described above with the upright "T" and the other half with the inverted "T" as the heat-associated pattern.

### Performance Index

The whole sequence of pattern motion for each fly was digitally recorded by a computer (sampling frequency: 20 Hz).

Performance indices [preference index before, avoidance index (AI) during and learning index (LI) after training] were calculated for each flight period as  $PI = (t1 - t2)/(t1 + t2)$ , with  $t1$  and  $t2$  indicating the time when the fly spent fixating the no-heat- ( $t1$ ) and heat-associated quadrants ( $t2$ ), respectively (38). Values of PI range from  $-1$  to  $1$ , with  $0$  indicating no pattern-specific preference,  $1$  indicating preference for the non-heat patterns during 100% of the time, and  $-1$  indicating continuous preference of the punishment-associated objects.

The pattern fixation index (PFI) is defined as the maximal absolute PI ( $|PI|_{max}$ ) of the three PIs in pretraining periods. The PFI is a measure of the fly's ability to stabilize the rotational movements of the arena (13,40), i.e., to fly straight, and reflects indirectly the fly's ability to perceive and to discriminate the visual objects presented in the learning paradigm.

As a measure for the flies' ability to perceive and discriminate the visual patterns used in the experiments, amplitudes of the second and fourth harmonics of the position histograms (averaged in Fig. 1) have been calculated by Fourier analysis (14) for single flies, averages of which are shown in Fig. 5.

### Significance Tests

Error bars in all figures indicate standard errors of the means (SEMs). Samples ( $n$ ) for experiments indicate the number of flies tested. Statistical significance of differences among three or more means are assessed with analyses of variance (ANOVA). Comparisons between the two means were assessed with Student's  $t$ -test.

## RESULTS

### Conditioned Visual Flight Orientation in Wild-Type Flies

In the flight simulator, the rotational movements of the panorama are made negatively proportion to the fly's yaw

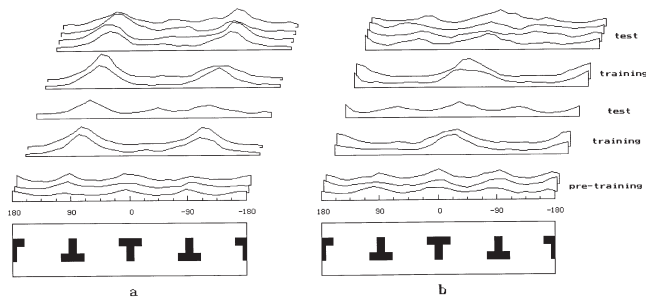


FIG. 1. Histograms of the relative time spent on different flight directions of *Drosophila*. (a) Thirty test flies were conditioned in the flight simulator to avoid orientations toward the upright "T"s. Whenever an upright "T" entered the frontal 90° sector of a fly's visual field, the heat reinforcement was on during training. (b) Another 30 flies were conditioned accordingly to avoid orientation toward the inverted "T"s. The projections of the panorama are shown below. The series of histograms above show the particular attraction of the inaccessible patterns on the panorama. The almost complete equidistribution of visits in the pretraining session results from the superposition of individual preferences not only in a choice between identical patterns (two upright or two inverted "T"s) but also in a choice between different patterns (upright vs. inverted "T"s). In the training periods, the flies avoided the heat-shock associated patterns either the upright "T"s (a) or the inverted "T"s (b). The "punishment" was switch off in the test periods. The retention of the avoidance was a significant effect in both sets of experiments. For description of the standard conditioning procedure, see the Method section.

torque. By this, the fly is enabled to choose its flight direction with respect to the visual landmarks.

With the standard conditioning procedure, 60 wild-type "Berlin" flies were investigated. Depending on the time spent by the flies on the different directions of flight, Fig. 1a and b show, in temporal sequence from front to back, the single phase of the standard conditioning procedure, obtained by evaluation of about 12 h of flight from 30 flies. The scale is 2 min per histogram. During the pretraining periods, a single fly spontaneously prefers either the "T" or the inverted "T." Sustained spontaneous alternation among the patterns tends to equalize the number of visits per pattern on the panorama. The equidistribution of choices may conceal the ability of a fly to discriminate among these patterns. The apparent ability to discriminate among different targets seemed to be similar in the present and in the previously reported experiments (12, 14). Association of the patterns of "T" (Fig. 1a), or the inverted patterns of "T" (Fig. 1b), with heat reinforcement during the training periods accounts for the avoidance of these patterns. The after-effect of this treatment is determined during the test periods. When the heat shocks were switched off in these periods, the flies still preferred a flight course away from patterns that were representing the negative reinforcement. Such conditioned visual flight orientation results from an associative process. Throughout the processes of experiments, the flies possessed the ability of flight control and "learning by doing," and were quickly and efficiently conditioned to prefer certain flight orientations to others.

Fourier analysis decomposes such histograms into two important constituents (14): (a) the amplitudes of the fourth harmonic with its peaks at  $0$ ,  $\pm 90$ , and  $\pm 180^\circ$  describe the attraction of the objects in comparison to void areas of the panorama. This component represents object perception; (b) the amplitudes of the second harmonic with its peaks at only two distinct positions of the panorama describe the individual preference of one object over the other. This component represents object discrimination (Fig. 5).

### Visual Associative Memory Is Disrupted by Exposure to BAL

The L-group of 44 flies, E-group of 46 flies and A-group of 30 flies respectively were exposed to BAL as described in the Method section. For each of these flies, learning acquisition and memory retention was tested in flight simulator. Sixty naive flies without any previous exposure to BAL (C-group) were used as control. Figure 2 shows the performance indices of the different groups in all periods.

The flies in all groups avoided the heat-associated objects about equally well. The AIs obtained during the four training periods of the four groups in Fig. 2 were significantly similar. A two-way ANOVA, with treatment and training period as main effects, indicated that the BAL treatment yielded no significantly different effects on the AIs,  $F(3, 176) = 0.29$ ,  $p > 0.1$ , whereas the sequence of the training periods produced a significant increase in the AIs,  $F(3, 176) = 2.82$ ,  $p < 0.05$ . The increase indicates that (a) learning itself may be involved in operant training, because the continuous improvement of the flies' performance as training proceeds, and (b) BAL has no deteriorating effect on the ability of the flies to sense heat reinforcement and to learn the association between heat and patterns.

Although after the first 4-min training periods the LIs are still small and not significantly different from zero in all four groups, in the test period of the second training session the C-group ( $LI = 0.3208 \pm 0.0445$ ) performed significantly bet-

ter than any other group (LIs =  $-0.008 \pm 0.0472$ ,  $0.0495 \pm 0.0643$ , and  $0.0879 \pm 0.0605$  for A-group, E-group, and L-group, respectively). These results clearly show that BAL has abolished almost completely the visual associative memory retention, when the flies were exposed to BAL in one of the three different developmental stages.

#### Gradually Recover From Amnesia

In an earlier study, we found that poor nutrition causes complete amnesia with three or four generations (13). The reverse shift from poor to nutritious food restores memory ability. Therefore, in the next experiment we investigate the ques-

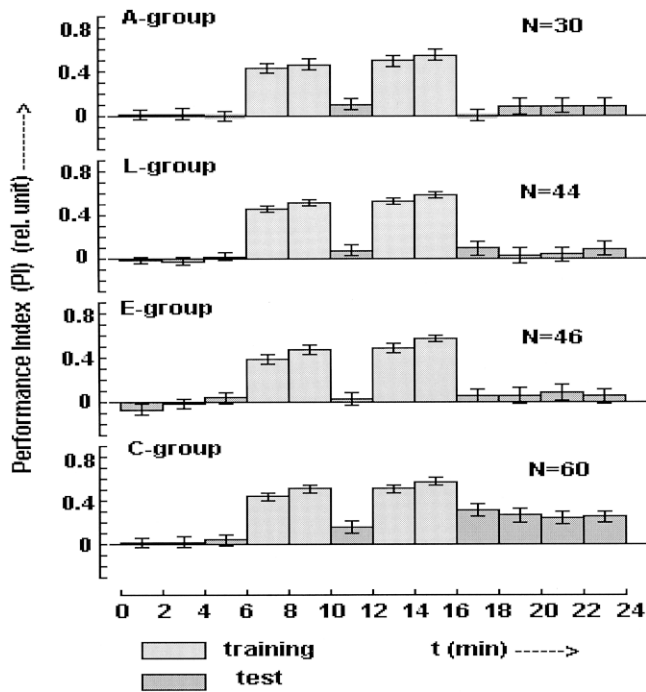


FIG. 2. Mean performance indices (PIs) of a number (N) of A-group, L-group, E-group, and C-group flies were obtained during a standard conditioning procedure. PIs include preference index before, avoidance index (AI) during and learning index (LI) after training. A-group, E-group, and L-group flies are exposed to benzaldehyde (BAL) in their adult, embryonic and larval stages, respectively. C-group flies are without any exposure to BAL groups (for detailed definition of different groups see the Method section). Flies were first tested for their spontaneous patterns preference (0–6 min) and subsequently conditioned with by heat to avoid two identical of the four patterns (6–10 and 12–16 min). Learning of safe flight orientations was assessed during the test periods (10–12 and 16–24 min). Error bars denote the SEMs. Data of C-group from Fig. 1. Flies of A-, L-, and E-group avoid heat-associated flight orientations but show almost no retention. To minimize the effect “active” memory decay caused by repeated testing, the LIs in the test period of the second training session were used for mutual comparison. One-way ANOVA revealed a significant effect on LIs in the test period of the second training session among groups,  $F(3, 176) = 7.39$ ,  $p < 0.01$ . Student's  $t$ -tests confirmed that the learning retention in the L-group, E-group, and A-group after the training is near zero, and significantly lower than that of the C-group,  $t(88) = 4.51$ ,  $t(104) = 3.60$ ,  $t(102) = 3.17$ ;  $p < 0.001$ , respectively, for LIs of the A-group, E-group, and L-group vs. that of the C-group.  $n$ : number of flies; error bars are SEMs.

tion whether amnesia caused by exposure to BAL may vanish, as well.

Successive generations (first and second) of the E-group flies were raised on noncontaminated medium, before they were subjected to the standard conditioning procedure. The control experiments were carried out on successive generations of C-group flies in almost same time. The LIs of all experimental groups in the test period of second training session are shown in Fig. 3. The means and SEMs on the left (0th generation) are taken from Fig. 2. Student's  $t$ -test confirmed that the LI of E-group was significantly lower than that of the C-group (see legend of Fig. 2). The first-generation flies performed better than the E-group flies, but the LI of them is not still different significant from that of the E-group. The LI of second-generation flies already approached the level of the LI of the control groups,  $t(38) = 0.37$ ,  $p > 0.05$ , and it differs significantly from the E-group,  $t(64) = 1.71$ ,  $p < 0.05$ . These results showed, that the E-group flies recovered from BAL-induced amnesia after at least two generations of rearing on a BAL-free medium.

The pattern fixation indices, or PFIs of A-group, E-group, L-group flies, which were treated with BAL before the training, were compared with the PFIs of the corresponding C-group flies (Fig. 4). One-way ANOVA revealed no significant differences in the PFIs of the different groups,  $F(3, 176) = 1.10$ ,  $p > 0.05$ . Although this is not a direct proof, BAL does not seem to affect the flies' ability of perception and discrimination of the visual objects. For a more rigorous proof, Fourier analysis was used and shown in Fig. 5. The symbols indicate the classification of the flies within the C-, E-, A-, and L-group according to their spontaneously preferred object. The means and SEMs of the harmonics represent the perception of the four objects, or the discrimination among these objects. Therefore, as a consequence from exposure to BAL, these relevant functions of the visual system seem not to be significantly disturbed.

#### The Level of cAMP Is Not Affected by BAL Treatment

Analysis of memory formation in learning and memory mutants after olfactory associative learning has suggested that

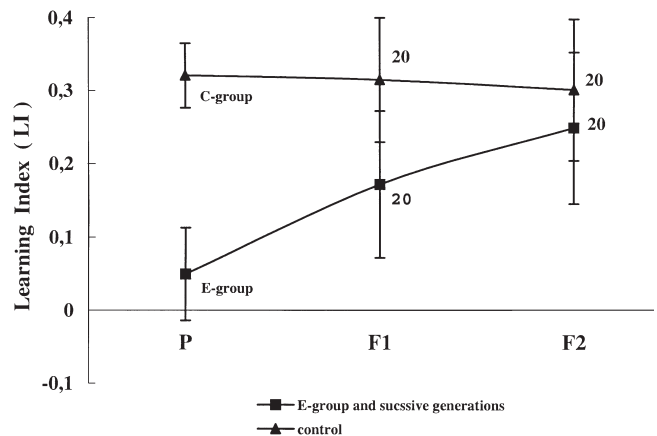


FIG. 3. Learning index (LI) of two consecutive generations of E-group flies raising on noncontaminated medium. After two generations of E-group flies raising on normal medium, the LI of the second generation rise to  $0.2492 \pm 0.1036$ . It differs significantly from the E-group,  $t(64) = 1.71$ ,  $p < 0.05$ , and does not from control group [LI =  $0.3013 \pm 0.0969$ ;  $t(38) = 0.37$ ,  $p > 0.05$ .  $n = 20$  flies per group and generation. The data on the left (0th generation) were derived from the results in Fig. 2.  $n$ : number of flies; error bars are SEMs.

early memory formation is disrupted in dunce and rutabaga mutants (9,30,33). Molecular and biochemical studies of dunce and rutabaga mutants revealed that both of them have abnormal levels of cAMP, which plays an important role underlying learning and memory in *Drosophila* (2,6,25,32). The deficiency of early memory formation in BAL-treated flies is very similar to that of dunce and rutabaga. Therefore, the question is very suggestive as to whether like causes could produce like effects in both the mutants of the cAMP cascade and the BAL-treated flies.

The amount of cyclic AMP in the heads of different treatment groups were measured using a radioimmunoassay. The results show that the heads of normal flies yielded about  $1.43 \pm 0.13$  ppmol of cyclic AMP per mg in adult heads (the number of extracts assayed is six). The E-group, L-group, and A-group of flies yielded, respectively,  $1.89 \pm 0.17$ ,  $1.51 \pm 0.17$ ,  $1.61 \pm 0.12$  ppmol per mg in adult heads, and the number of extracts assayed are 10, 8, and 10, respectively. It shows that the level of cyclic AMP in heads of different group flies have no significant difference,  $F(3, 30) = 1.68$ ,  $p > 0.05$ . The levels of them are significantly different from dunce mutants [ $2.3\text{--}2.8$  ppmol per mg for detail see (2)]. It suggests that the BAL treatment does not affect the level of cAMP in flies' heads.

#### DISCUSSION

In the flight simulator, the test fly is held in a fixed orientation by the yaw torque meter. The fly's attempted turns are transformed into counterrotations of the surrounding cylindrical panorama. This allows the fly to maneuver the preferred object into the actual direction of flight. Context-dependent performance in a choice among different visual objects of otherwise identical appearance are recorded during stationary flight of the fly in the flight simulator.

The heat-conditioned visual object discrimination suggests a significant association between the heat and the particular features of the visual objects. Behavioral performance of the flies in the flight simulator is highly variable and the performance index of single fly does not reflect the group average, although the causes of this variance are not yet completely understood (13). Nevertheless, averaged performance of the flies in Fig. 1 indicates that the flies can avoid the heat-associ-

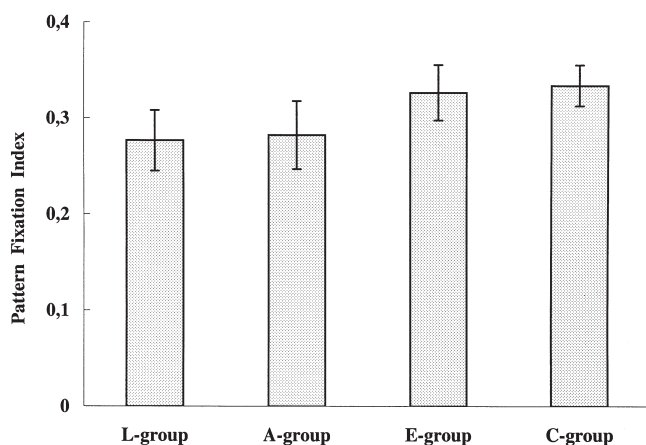


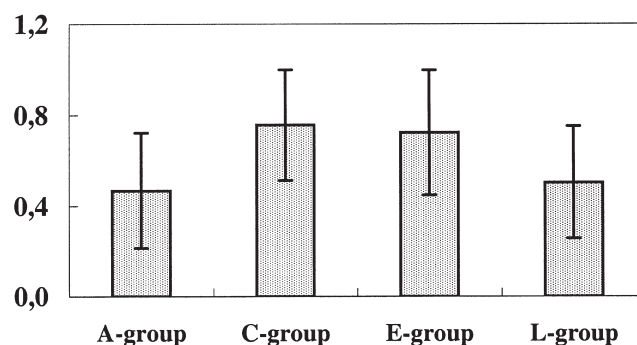
FIG. 4. Pattern fixation indices (PFI) of flies of the four different treatment groups (for detailed definition of PFI see the Method section). BAL does not seem to interfere with the perception and discrimination of visual objects.

ated patterns during training, and continuously avoid flight directions towards the visual object associated with the heat during the following test without any heat.

In the flight simulator the flies are capable of learning and memory. However, the avoidance behavior itself is an operant behavior (38), and there is no evidence that learning processes are involved in operant behavior (36). But, if flies increase their avoiding indices with increasing training time, this increment may be interpreted as a reference to learning. Figure 2 shows that normal flies (C-group) continuously improve their

#### Spontaneous object discrimination

##### Direction histogram, 2<sup>nd</sup> harmonic



#### Spontaneous object perception

##### Direction histogram, 4<sup>th</sup> harmonic

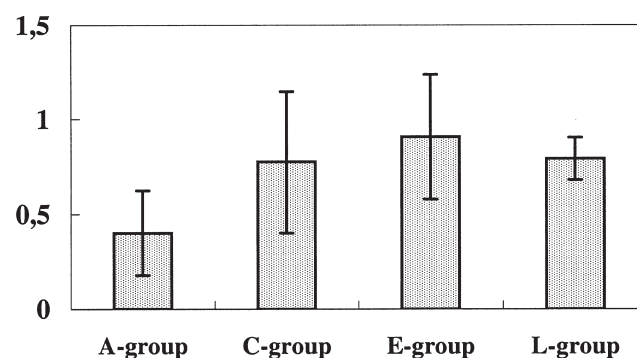


FIG. 5. Two essential constituents of the pretest histograms from the flies of the C-, E-, A-, and L-groups in Fig. 3 were derived by Fourier analysis. The amplitude of the fourth harmonic with its peaks at the positions of any of the visual objects ( $0, \pm 90$  and  $\pm 180^\circ$ ) represents the ability of perception of the four objects. The amplitude of the second harmonic with its peaks at the positions of the spontaneously preferred objects and their trough at the positions of the spontaneously rejected objects reflects the fly's ability to discriminate the two different pattern types (upright and inverted T). The corresponding diagrams show the means and SEMs of these amplitudes in units of the normalized mean value of the histograms. The results are given in blocks. They show the means of contributions of flies with a preference for either the upright or inverted "T." Neither the perception nor the discrimination of the two objects is significantly impaired by the early uptake of response-inhibiting amounts of the repellent BAL.



behavioral performance as the training proceeds. During test sequences, the performance indices of normal flies (C-group) are significantly larger than zero. This demonstrates that the flies are capable to memorize. This finding is supported by the observation during the experiments, that frequently the time course of the fly's yaw torque shows outbreaks of violence (i.e., increase of frequency and amplitude), whenever it turns towards an object that was associated with the heat during training (data not show). Other flies turn quickly back from the objects associated with heat, with increased angular velocity.

Several nonassociative learning paradigms have been studied with *Drosophila* (3,10,21). Flies that had been reared on a medium containing a repellent odor showed reduced aversion to the odor in the adult stage. It has been shown that this change is caused by a form of habituation (21). This nonassociative modification suggests that the prolonged exposure to an odorant may decrease the animal's sensitivity to a chemical substance. In the flight simulator using BAL as a negative reinforcement, flies exposed to BAL in different developmental stages seemed to become indifferent to BAL (14). Lacking proper controls for nonassociative changes, habituation could not be ruled out as a possible cause in odor associative learning. Therefore, further experiments were carried out in our learning paradigm using heat as negative reinforcement. As shown in Fig. 2, all flies exposed to BAL produced about the same avoidance indices as the control flies during training. This indicates that these flies avoid the heat-associated pattern efficiently, associate this pattern with heat, and improve their avoidance behavior by learning in a preceding training as well as normal wild-type flies. After training, the flies are tested for memory formation. The results show clearly that the C-group flies have a significantly higher level of retention in the test periods than all of the other groups. Exposure to BAL in the adult, larval, or even embryonic stage abolishes the visual associated memory in *Drosophila*.

Apparent amnesia as a consequence of disturbed vision and sense can be dismissed. In the present experiments, the flies showed normal spontaneous pattern preference during the pretraining periods. Figure 4 shows that BAL did not significantly affect the PFIs of the different treatment groups of flies, because the flies were able to stabilize the panorama or to choose their preferred pattern. In Fig. 5, Fourier analysis was applied to show further that neither object perception nor object discrimination of the flies exposure to BAL is altered. So far, we can exclude substantial inhibitory effects of BAL on the perception and discrimination of visual patterns (14), on sensing heat reinforcement, and on associating heat with the "punished" pattern. It suggest that exposure to BAL may interfere directly with the memory formation in *Drosophila*.

The mechanism by which the uptake of BAL may affect the associative memory in *Drosophila* is not yet clear. Although the levels of cAMP were not changed in the heads of different groups that were treated with BAL, we cannot exclude an important role of the cAMP signaling pathways. Alkylxanthines, cyclic nucleotide phosphodiesterase inhibitors, when fed to wild-type adults of *Drosophila* adults, can cause memory decay after training with the unchanged level

of cAMP in flies' heads (1). The various tasks may be a consequence of differences in the biochemical machinery (23). Intracellular cAMP levels can be viewed as a "memory trace in time and space" of the history of previous synaptic activation (22). BAL may interfere with the dynamics of cAMP at several points of the cAMP metabolism.

The biochemical and molecular machinery by which BAL affects memory retention in associative learning and memory is still unknown. Exposure to benzaldehyde in adult, larval stage, or exclusively in their embryonic states as eggs in the ovaries of virgin females fully abolished the retention of learning in the adult flies. However, the amnesia is recoverable, after the flies are shifted to normal medium. This seems to suggest that BAL may affect the development of the brain in *Drosophila*. In many species environmental factors trigger different developmental programs (19,20). The fly food has also an influence on learning (35). Poor nutrition causes complete amnesia within three or four generations. The reverse shift from poor to nutritious food restores learning ability within an even longer delay (13,40). During development, metamorphosis in Diptera is accompanied with histolysis and larval tissue reconstruction of body sense organs, but the central nervous system is less affected (21). Most of the adult motor functions and neuromodulatory processes may derive from neurons of larval origin (34). Honey bees combine visual input from optic lobes and olfactory input from the antennal lobes in their mushroom bodies (MBs) in a complex scheme of context-dependent modal integration (11). The processing of olfactory information has been investigated in considerable detail in *Drosophila* (27). The experiments of MB mutants and the complete and precise MB ablation using hydroxyurea during early development demonstrated that the MBs are specialized structures mediating olfactory learning and memory processes in flies (7,15,16). In the olfactory system of *Drosophila* the uptake of odor such as benzaldehyde, and its transformation into an odor-specific pattern of activated "glomeruli" in the antennal lobes and the calyces of the MBs has been demonstrated (4,28). MBs are assumed to play a role in the processing and storage of chemosensory information. In various insect species, the calyces of the MBs also receive fibers from other sensory systems and are, therefore, likely to processes multimodal information (11,29). The MBs of *Drosophila* show a remarkable degree of plasticity, both during the course of their development and in response to environmental stimuli received by the adult flies (29). The effect of early exposure to BAL on the performance of the flies might reflect the plasticity of the MBs during their development.

So far, a valid explanation for the effect of BAL requires further experiments. It appears worthwhile to explore the unknown neuronal, physiological, and biochemical basis behind the long-term effect of BAL.

#### ACKNOWLEDGEMENTS

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