J. comp. Physiol. 114, 69–89 (1977)

Journal of Comparative Physiology · A

Properties of Learning and Memory in *Drosophila melanogaster*

Yadin Dudai*
Division of Biology, California Institute of Technology, Pasadena, California 91125, USA

Received September 20, 1976

Summary. *Drosophila melanogaster* can be conditioned to avoid an odorant selectively after being shocked in its presence (Quinn et al., 1974). In the following study learning and memory properties of the flies are reported. The major part of the conditioned behavior is acquired after a single training trial (Fig. 2). Similar degrees of learning are obtained by using various odorants in various combinations (Table 1). The flies can learn to avoid selectively several odorants at a time, can learn to discriminate between different concentrations of the same odorant (Fig. 4), and can also learn to distinguish a mixture of odorants from its components. If not extinguished, the selective avoidance decays slowly and can be detected for hours, its magnitude depending upon the intensity of training (Fig. 6). Memory can be disrupted by narcosis during the first ~20 min after training, but not afterwards (Fig. 7). A study of learning properties of wild-type strains and various morphological and behavioral mutants reveals differences in performance (Table 2). However, the differences cannot be attributed with certainty to differences in learning and memory, per se, because the mutants differ in other aspects of behavior, e.g., locomotor activity and phototaxis. Of the wild-type strains tested, Canton-S performed the best.

Introduction

The mechanism of learning and memory can be studied by producing genetic lesions which specifically interfere with these processes, and trying to elucidate the anatomical and the molecular defects caused by the mutation (Benzer, 1973). *Drosophila melanogaster* is a convenient model organism for such studies. It can learn (Quinn et al., 1974; Spatz et al., 1974), and is readily amenable to genetic analysis.

* Present address: Department of Neurobiology, The Weizmann Institute of Science, Rehovoth, Israel

The interpretation of conditioning experiments in *Drosophila* and other dipterans is complicated because of phenomena such as sensitization and pseudoconditioning (Dethier, 1966), habituation (Manning, 1967) and odor cues left in the instruments by the flies during training (Yeatman and Hirsch, 1971; Murphey, 1973). Using appropriate controls Nelson (1971) demonstrated classical conditioning, based on gustatory cues, in the blowfly, *Phormia regina*; and Quinn et al. (1974) demonstrated that populations of *Drosophila* can be conditioned to avoid odorants (or colors) after being repeatedly shocked in their presence.

In the following, properties of conditioned behavior based on chemoreception in *Drosophila* are described and analyzed. Among the questions posed at the beginning of the study were: How fast and to what degree can flies learn to avoid a shock-coupled compound, and what part of the observed avoidance can be attributed to the selective association of the negative reinforcement with the chemical cue? Can flies store multiple items of information, and what are some of the discriminative properties of their learning ability? How long can flies remember, can memory be disrupted, how and when? And do different strains and morphological and behavioral mutants differ in their learning ability?

One of the objectives of this study is to serve as groundwork for further studies, in which the learning process in *Drosophila* will be dissected by the use of specific mutations. A mutant, *dunce,* deficient in learning, has been recently isolated (Dudai et al., 1976).

Materials and Methods

Flies. Drosophila melanogaster of the Canton-Special (C-S) strain were used as normal flies. All the stocks used were kept in the collection of Dr. S. Benzer at the California Institute of Technology, Pasadena, and were cultured under standard conditions (Lewis, 1960). The mutants C29 Hlcnbw/SM5, 1(2)Ddc^{n1}/Cy0, and 1(2)Ddc^{n2}/Cy0 were kindly provided by Dr. T. Wright, University of Virginia, Charlottesville. X-linked mutations in a hemizygous state were kept over *yf*X̂X females. The latter were previously crossed to replace their autosomes with C-S material. When autosomal mutations were tested in the heterozygous state, the parental strains were crossed to C-S to obtain mutation/+ flies. Studies were made using 2- to 5-days old flies. When there was a need to separate flies prior to experiments (e.g., mutant males from *yf*X̂X females), N_2-narcosis was routinely employed (see below), and the flies were tested 1 to 4 days after the treatment.

Chemicals. 3-octanol, 4-methylcyclohexanol, and geraniol were obtained from K & K Laboratories (Plainview, N.Y.), benzaldehyde, amyl acetate and menthol were from Mallinckrodt Chemical Works (St. Louis, Mo.), caproic acid from National Biochemical Corp. (Cleveland, Ohio), and stearic acid from Metheson, Coleman and Bell Inc. (Norwood, Ohio). Cycloheximide was purchased from Sigma (St. Louis, Mo.). All other chemicals were of analytical grade.

Learning Apparatus. The apparatus originally designed for behavioral countercurrent distribution (Benzer, 1967) and later employed for learning experiments (Quinn et al., 1974) was used (Fig. 1). Plastic tubes, 17 × 100 mm (No. 2017, Falcon Plastics, Oxnard, California) were aired for at least 24 h and were used for one experiment only. "Rest" tubes, prepared by puncturing holes in the end of the plastic tubes, and grids made from printed-circuit material, were as described by Quinn et al. (1974) except that the stripes on the grids were 0.5 mm wide, 1 mm apart, and were arranged parallel to the grid's tabs (Fig. 1). Grids with the latter pattern were found to be easier to handle, as they stay flat after washing, in contrast to grids with stripes perpendicular

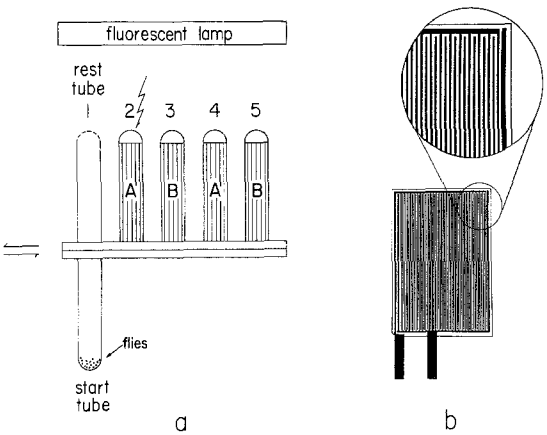

Fig. 1. a Apparatus used for training and testing flies. Two plastic blocks can be slid past each other on a dove-tail joint. Holes running through each block are fitted with Teflon 0-rings, to grip plastic tubes. Tube 1 is the rest tube, and is perforated at the closed end to allow fresh air flow. In the standard paradigm, tubes 2 and 3 are used for training, and tubes 4 and 5 are used for testing. Tube 6 is the start tube, and can be shifted in register with each of the other tubes. Vertical strips in tubes indicate grids. *A* and *B* indicate two odorants, e.g., 3-octanol and 4-methylcyclohexanol. Voltage (indicated by broken arrow) is applied either to tube 2 or to tube 3. When more than two odorants were used in a single experiment, the flies were trained in one apparatus and tested in another one. See text for training and testing procedures. **b** Printed circuit grid for shocking flies. The grid is rolled up and inserted into a plastic tube, which is plugged into the apparatus. Conductive tabs for applying voltage are bent around the tube rim to the outside. Figure adopted from Dudai et al., 1976

to the tabs, which acquire the shape of the plastic tube. Grids were cleaned before the experiments by two 16-h washes in absolute ethanol, followed by successive rinsing in distilled water, absolute ethanol, and ethyl ether. The grids were then aired inside a hood for at least 20 min. Solutions of compounds to be used in the experiment were always made in absolute ethanol. The solution (0.2 ml) was spread over the grid with a pipette, and the ethanol was allowed to evaporate for about 5 min at 24 ± 2 °C, $40\pm10\%$ relative humidity. Each grid was then rolled up and inserted into one of the plastic tubes. The conductive tabs for applying voltage were bent around the tube rim to the outside. Grids so prepared were routinely used for no more than 90 min. The experiments were carried out in a darkened room at 22 ± 2 °C, $45\pm10\%$ relative humidity. A 15 W fluorescent lamp (Westinghouse, daylight F15T8/D) was the light source for phototaxis. The shock reinforcement was applied to the grids through a transformer. Unless otherwise indicated, a shock of 90 V (60 Hz) was employed.

Training. 35 to 45 flies were used for each experiment. Unless otherwise indicated, each population was used for one experiment only. The flies were transferred to fresh food bottles, to allow them to clean themselves, for 10 to 60 min before training. Prior to the run the flies were transferred into a fresh plastic tube ("start tube") which was inserted in the apparatus in register with the rest tube. The flies were then allowed to explore the tubes and to clean themselves again for 1 min. The run was started by holding the apparatus vertically and tapping it on a rubber pad to shake the flies to the bottom of the start tube. The apparatus was then laid horizontally before the lamp, at a distance of 2 to 3 cm from the tips of the tubes. Driven by their phototactic response, the flies ran into the rest tube. After 15 s, the flies in the start tube were counted and after an additional 30 s, all the flies were shaken into the bottom of the start tube, and the latter was shifted into register with the appropriate grid tube. The apparatus was then again laid horizontally before the fluorescent lamp, and the flies ran into the appropriate tube. The

flies in the start tube were again counted after 15 s, and after 30 s all the flies were shaken again into the start tube, shifted into register with the rest tube for 30 s, and so forth. A training cycle was thus composed of 30 s runs into the grid tube(s), with 30 s runs into the rest tube in between, in each case recording the number of flies in the start tube (i.e., those not entering the rest tube or the odorant tube) 15 s after the start of the run. A period of 15 s was chosen because this is the time required for 98% of a population of normal flies to enter the rest tube. The exact sequence of runs in each training cycle and the number of training cycles depended on the specific experiment, as described under Results.

Testing. The flies were always tested in new tubes, not the tubes in which they had been trained, in order to eliminate any cues which they might have left during the training. The test was performed in a sequence similar to that of a single training cycle but with no shock associated with any of the odorants. Thus, following a run into the rest tube, the flies were shifted to a new tube with grid and odor, and the number of flies avoiding the grid was counted after 15 s; the flies were then shifted again to the rest tube for 30 s, and then into the second odorant. When more than two odorants were used in one experiment, the sequence was continued. After each experiment was completed the flies were etherized and the total number counted.

Behavioral Indices. Several quantitative indices were found useful in analyzing the behavior of the various strains and mutants during training and testing:

a) The *learning index* is a measure of the specific odorant avoidance acquired during training as defined by Quinn et al. (1974); i.e., the fraction of the population avoiding the shock-associated compound during testing minus the fraction avoiding the control compound. In experiments in which the flies were trained to avoid one shock-associated compound in each reciprocal half of the experiment, a learning index (denoted by λ_i) was obtained for each reciprocal half, and the overall learning index of the complete experiment, Λ, was the average of the two values ($\Lambda = (\lambda_1 + \lambda_2)/2$). In experiments in which the flies were trained to avoid several compounds concomitantly, λ_i was separately calculated for each of the shock-associated compounds in each reciprocal part of the experiment, and Λ was calculated as the overall mean ($\Lambda = \Sigma\lambda_i/n$, $i = 1 \ldots n$). A value of $\Lambda = 0$ represents no learning, $\Lambda = 1$ represents perfect learning and $\Lambda = -1$ indicates perfect "masochism".

b) The *nonphototaxis index,* a measure of the general inactivity and lack of phototactic response of the flies during testing, is defined as the fraction of flies which do not enter the rest tube during the test. Usually the flies were run into the rest tube more than once during the testing cycle (e.g., once at the beginning of the test, and again between testing the avoidance of the control compound and that of the shock-associated compound); the nonphototaxis index was then calculated as the average for all the runs into the rest tube during the test. Values can range from 0 to 1, with 0 indicating complete phototaxis and 1 indicating complete lack of phototaxis.

c) The *avoidance index* is defined as the fraction of flies avoiding the shock-associated compound during the last training cycle. An index of 1 indicates complete avoidance of the electrified tube and 0 indicates no avoidance at all.

d) The *control-avoidance index* is defined as the fraction of flies avoiding the control compound during the last training cycle. Again an index of 1 indicates complete avoidance of the control compound and 0 indicates no avoidance at all.

Memory Experiments. In these experiments the flies were not tested immediately following training, but only after an appropriate interval, as described under Results. For intervals less than 1 h the flies were stored in the start tube, which was removed from the apparatus and closed with a foam rubber stopper. For intervals longer than 1 h the flies were stored in vials containing culture medium (Lewis, 1960). The flies were transferred into a fresh tube 1 min before the test.

Narcosis. a) *Nitrogen narcosis:* N_2-narcosis was employed for separation of flies prior to training and testing (Byers and Quinn, 1974), and for memory disruption experiments. In the latter, flies were introduced after training into an empty culture bottle, with a foam rubber stopper, and a gentle stream of N_2 was introduced for 5 min. The flies passed out after about 45 s. When removed from the N_2, they recovered in about 3 min. The flies were then kept in a plastic tube, with a foam stopper, under room light. Prior to testing they were transferred into a fresh tube.

b) *Cold narcosis:* Cold narcosis was used in memory disruption experiments. A plastic tube containing the flies and stopped with a foam stopper was inserted into an ice bucket, and the flies were gently shaken to the bottom of the tube. They passed out after 45 s. Five min later the tube was removed from the ice and the flies transferred into a new plastic tube. They began to recover in about 50 s and were kept in the tube until training.

Statistics. All results are presented as the mean and standard error of the mean. When significance levels are indicated, they are based on Student's t-Test (Bailey, 1969).

Results

A. Learning

1. Acquisition of Avoidance of Shock-coupled Chemicals

Flies were electrically shocked while presented with a single odorant and were then tested for their avoidance of the same odorant, without shock, and of a control odorant which had not been presented during training. As a control for odor bias, another population of flies was shocked in the same apparatus, but this time the former control odorant served as the shock-associated odorant and vice-versa. In a typical set of experiments using 0.5% 3-octanol and 0.5% 4-methylcyclohexanol as odorants, $59 \pm 1\%$ of the flies avoided the shock-associated grid at the end of the first training cycle. When tested with new grids after this single training cycle, $55 \pm 2\%$ of the flies avoided the shock-associated odorant, whereas $20 \pm 3\%$ avoided the control compound (the values are averages for 32 experiments each). A learning index (A) of 0.35 was thus obtained. Increasing the number of training cycles to 3, and testing immediately afterwards, led to an increase of the learning index to 0.54 ($76 \pm 2\%$ of the flies avoided the shock-associated odorant during the last training cycle; $70 \pm 3\%$ avoided it during the test, whereas $16 \pm 3\%$ avoided the control odorant during the test). A further increase in the number of training cycles did not improve the learning index (Fig. 2).

Similar results were obtained when using other chemicals as cues (see below), and with shock voltages of 20 V, 50 V, 90 V, or 140 V (AC, 60 HZ). When only 5 V was used, the avoidance was less than half of the values mentioned above. When using 140 V some of the flies became temporarily damaged and rolled on their backs in uncoordinated fashion in the start tube for 10 to 20 s after the shock. Only rarely were such symptoms observed with lower voltages. A shock of 90 V was routinely used in all the following experiments.

2. Components of Avoidance

In the experiments described above, the flies were presented during training with only one compound, associated with shock, and later tested for their avoidance of the same compound and a new one. It is necessary to establish what part of the selective avoidance behavior is indeed due to the negative reinforcement provided by the electric shock. Flies were therefore presented with a compound and tested for avoidance as above, but without being shocked electrically during training. Even so, the flies display during the test an apparent "learning index" measuring from 0.08 ± 0.03 (after one "training" cycle) to 0.20 ± 0.04 (after three or more cycles) (Fig. 2A).

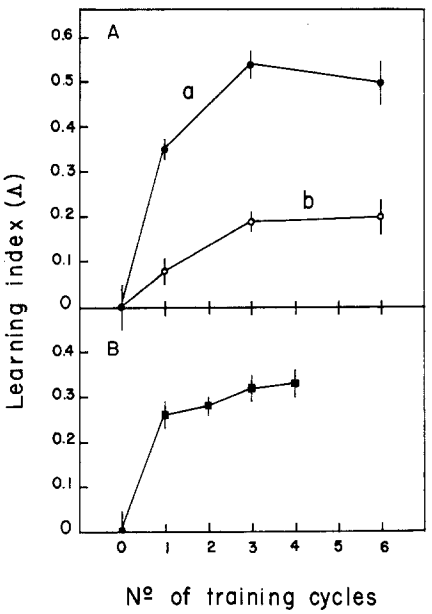

Fig. 2A and B. Acquisition of selective avoidance to a compound as a function of the number of training cycles.
A Flies presented with only one compound during training, and tested for their avoidance of the same compound and of a control one. *a*, Electric shock associated with the compound during training. *b*, Control in which no electric shock was applied during training.
B Flies exposed both to the shock-associated compound and to the control-compound during training. The learning index is the fraction of flies avoiding the shock-associated compound minus the fraction avoiding the control-compound during the test, and is calculated as the average for the two reciprocal halves of an experiment, in which the shock- and control-compounds are interchanged. Results are for 6 to 12 experiments each

What is the origin of the increased avoidance obtained without electric shock? It is possible that the flies become sensitized to the compound presented repeatedly during training; or that the training procedure involves negative reinforcements other than the electric shock. For example, the paradigm utilizes phototaxis to drive the flies toward the grids, and rapid phototaxis is initiated by banging the flies to excite them (Benzer, 1967). Mechanical disturbance also occurs in returning the flies rapidly to the start tube. Such banging and agitation might themselves serve as a negative reinforcement.

When the banging is omitted, and the flies transferred from tube to tube by gently rotating the apparatus on the table to and from the light, phototaxis is poor and most of the flies do not enter the odorant tube. Even under these conditions a "learning index" of 0.09 ± 0.03 ($n=8$) is obtained when the flies are tested after a single 120 s presentation with an odorant (no shock). But negative reinforcement cannot totally be excluded because even slight tilting of the apparatus, hand waving or just confinement in a closed tube may serve as aversive stimuli. It is therefore impossible, with this experimental design, to dissociate possible sensitization from conditioning due to handling procedures.

3. Avoidance Due to Electric-Shock-Association Only

To eliminate the above complications and to isolate only the conditioning component due to the presentation of a chemical with electric shock, the paradigm described by Quinn et al. (1974) was used. In this paradigm the flies are presented during training with two odorants, one associated with the electric shock, the other not. The flies are then tested vs. both odorants. The paradigm is then repeated with a second population of flies, but the shock-associated- and control-

compounds are interchanged. Thus, any component of "learning index" due either to sensitization or to an uncontrolled negative reinforcement is cancelled by averaging the learning indices from the results of the two reciprocal halves of the experiment.

As shown in Figure 2B, a major part of the learning is displayed after a single training cycle. For routine work, unless otherwise indicated, a 3-training-cycle paradigm (defined as the "standard" paradigm) was employed. Note that the learning curve in Figure 2B essentially corresponds to the difference between curves a and b in Figure 2A.

4. Behavior during Training

During a multiple training-cycle experiment, the fraction of flies avoiding the shock-associated compound increases mainly after the first training cycle, but increases only slightly further, if at all, after the second cycle (Fig. 3). This is consistent with the result mentioned above that a major part of the conditioned behavior is acquired by the flies after a single training run. From the second training cycle on, many flies turn away from the shock-associated grid before reaching it.

The increase in avoidance during training is not necessarily due to an increase in learning. Flies avoid more a shock-associated odorant on their first run into it, if other flies have been previously shocked there (Fig. 3). Thus, at least part of the increased avoidance during training is due to some odorous cue left on the grid by the shocked flies. In order to test whether this cue, by itself, can serve as a negative reinforcement, the following experiment was performed: A group of flies had been repeatedly shocked (for three successive 30 s periods, with 45 s rest in between) on a grid with an odorant. Another

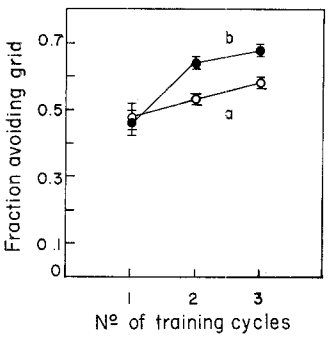

Fig. 3. Avoidance of a shock grid during training by flies repeatedly shocked on the same grid, and by naive flies. The grid contained either 3-octanol or 4-methylcyclohexanol. (*a*) Avoidance of shock grid by groups of naive flies. Data were calculated from 3 successive single-training-cycle experiments, and thus in each training cycle a new group of flies had been run into the same grid. The increase in avoidance of each new group is due to some cue left on the grid by the former group, and this cue may therefore account for part of the increase in avoidance seen in curve *b*. (*b*) Avoidance of shock grid by the same group of flies in the 3 successive training cycles of a standard paradigm. The average fraction of flies avoiding a non-shock grid with the same odorants on their first run into it was 0.16 ± 0.05. Each point represents 8 to 16 experiments

group of flies was then run (again for three successive 30 s periods) into the same grid and into a grid containing a control odorant. No shock was used in any of the cases. The flies were then tested for avoidance of each odorant. The reciprocal half of the experiment, i.e., interchanging the odors, was also performed. An average learning index of essentially zero ($A=0.03$) was obtained from four such experiments. This indicates that any cue left by shocked flies on the grid does not serve as a negative reinforcement for other flies. Such cues, however, might still serve as an additional reinforcement for the flies once they themselves have been shocked on the grid.

An important question is the degree of damage due to shock during training. With the routine 90 V shock the flies were often seen to fall and roll on their backs while on the shock-grid. One way to estimate the damage is to compare phototaxis into the rest tube before and after a shock step, during training. For C-S flies in a 3-training-cycle experiment, avoidance of the rest tube before shock was $(3\pm0.3)\%$ (180 measurements), and after shock it was $(6\pm0.4)\%$ (172 measurements). Thus although this difference is significant, the absolute decrease in phototaxis is very small.

5. Conditioning toward Various Chemicals and Some Discriminative Properties

Various chemicals can be used as cues in the conditioning experiments described above. These include acids, alcohols, aldehydes, and esters (Table 1). Some compounds did not work when applied to the grid (e.g., sucrose, fructose). Some chemicals which can be learned individually cannot be distinguished from one another in the paradigm (e.g., citric acid vs. tartaric acid). Most of the chemicals tested gave a learning index of 0.2 to 0.4, whether tested vs. another compound or vs. a blank grid, i.e., a grid to which only ethanol was applied and allowed to evaporate (Table 1). None of the compounds tested so far in the standard 3-training-cycle paradigm has given consistenly a learning index significantly higher than 0.4. Some of the compounds were technically more difficult to work with than others. Stearic acid, for example, forms a greasy layer on the grid which takes a long time to dry, and tartaric acid was not easily washed off. In routine work, unless otherwise indicated, 0.5% 3-octanol and 0.5% 4-methylcyclohexanol were used.

Using odorants listed in Table 1, some discriminative properties were investigated. *Drosophila* can learn to avoid one concentration of an odorant and not to avoid another concentration (Fig. 4). The learning indices were significantly lower ($P<0.01$) in those half experiments in which the higher odorant concentration served as control. This could be expected, since the flies tend to avoid higher odorant concentrations more than lower ones. In addition, one might expect the flies which are moving toward the higher concentration grid to reach a zone which has about the same concentration as they were exposed to when shock was administered. The observation that the flies do learn even under these conditions indicates that they can distinguish between the low-concentration grid and any zone along the odorant gradient which originates from the high-concentration grid. This is probably because the sensory

Table 1. Learning of various compounds by *Drosophila*

Compounds Tested		Learning Index
a	b	
ethanol	clean grid	0.04±0.03 (n=4)
3-octanol, 0.25%	ethanol	0.28±0.06 (n=4)
3-octanol, 0.5%	ethanol	0.38±0.07 (n=6)
4-methylcyclohexanol, 0.5%	ethanol	0.24±0.04 (n=6)
3-octanol, 0.5%	4-methylcyclohexanol, 0.5%	0.34±0.02 (n=9)
stearic acid, 1%	4-methylcyclohexanol, 0.5%	0.29±0.05 (n=3)
menthol, 0.25%	ethanol	0.24±0.06 (n=4)
geraniol, 0.05%	ethanol	0.30 (n=2)
tartaric acid, 1%	ethanol	0.42±0.05 (n=4)
tartaric acid, 1%	geraniol, 0.05%	0.43±0.05 (n=4)
citric acid, 1%	ethanol	0.26±0.01 (n=3)
citric acid, 1%	tartaric acid, 1%	0.02 (n=2)
caproic acid, 0.25%	ethanol	0.29±0.05 (n=3)
caproic acid, 0.25%	tartaric acid, 1%	0.24±0.03 (n=4)
acetic acid, 1%	n-decanol, 0.5%	0.25±0.08 (n=4)
fructose, 1.5%	ethanol	0.08±0.01 (n=3)
butylacetate, 0.5%	glycerine, 0.5%	0.15±0.06 (n=4)
amylacetate, 0.5%	ethanol	0.31±0.03 (n=4)
benzaldehyde, 0.5%	ethanol	0.28±0.04 (n=3)
amylacetate, 0.5%	benzaldehyde, 0.1%	0.30±0.06 (n=4)

Learning indices are for the standard paradigm. All solutions are in absolute ethanol. Values are mean±SEM; N=number of experiments

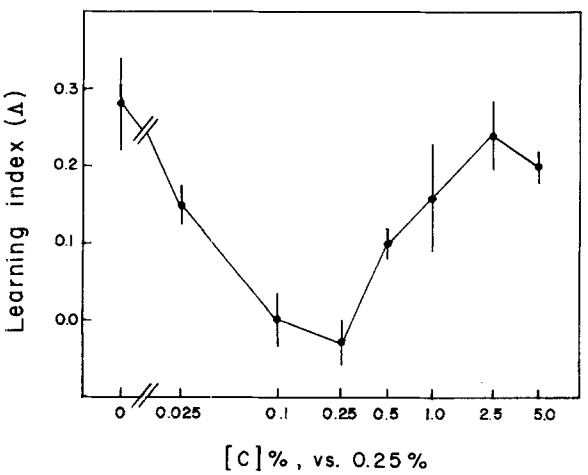

Fig. 4. The ability of flies to learn to avoid selectively one vs. another concentration of the same compound. Flies were presented with two concentrations of 3-octanol. One concentration was always 0.25% and the other, (C), was changed from 0 to 5%. Shock was associated with one of the two concentrations in each reciprocal half of each experiment, and the flies were trained and tested in the standard paradigm. Each point represents 4 experiments. The concentrations refer to the original odorant solutions applied to the grid, and are not necessarily proportional to the amount of odorant evaporated from the surface of the grid inside the tube

cue associated with the shock is composed of a combination of odors (or even tastes) of the grid and the chemical, and such a combination is not identical to the odor at any point along the odorant gradient which they sense while approaching the high-concentration grid.

The flies are also able to distinguish between on odorant and a mixture of that odorant plus others. Thus, they learned to avoid either 3-octanol or 4-methylcyclohexanol when a mixture of both odorants served as control, and vice versa, with an average learning index of 0.26.

6. Multiplicity of Information

To determine whether flies can learn to avoid selectively more than one out of several odorants at a time, they were trained to avoid one out of two, two out of three, or three out of four compounds in a single experiment. The flies were alternately presented with all the odorants (30 s for each odorant, with 40 s rest between odorants). All but one of the compounds presented were coupled to shock, the one compound serving as control. Following three cycles of training, the flies were tested for avoidance of each compound, and the individual learning indices, one for each shock-associated compound, were calculated. The experiment was then performed again with fresh groups of flies, each compound in turn serving as the control. Thus, in the entire experiment, each compound served as control in one part of the experiment, and as shock-associated in the other parts. The overall learning index was calculated as the average of all the individual indices.

For experiments in which the flies were trained to avoid one out of two compounds, 0.5% 3-octanol and 0.5% 4-methylcyclohexanol were used, and an overall A of 0.34 ± 0.02 ($n=9$) was obtained. For experiments in which the flies were trained to avoid two out of three compounds, the above compounds plus 1.0% stearic acid were used, and an overall A of 0.28 ± 0.04 ($n=4$) was obtained. For experiments in which the flies were trained to avoid 3 out of 4 compounds at a time, the above compounds plus 0.05% geraniol were used and an overall A of 0.18 ± 0.04 ($n=4$) was obtained. All shock-associated compounds can be learned in each experiment.

The decrease in the learning ability in the multiple-compound experiments is probably not due to fatigue, as no unusual decrease in phototaxis was observed. Nor is it due to diffusion away of the odorants during $1^1/_2$ h, the time required to complete one 4-compound experiment, since the instrument could still be efficiently used for training naive flies. It is more likely that the decreased A is due to limitations of the learning capacity of the flies, e.g., due to confusion.

B. Memory

1. Extinction

When the flies are repeatedly run into the testing tubes, following training, the learning index decreases with time (Quinn et al., 1974); however, the decrease

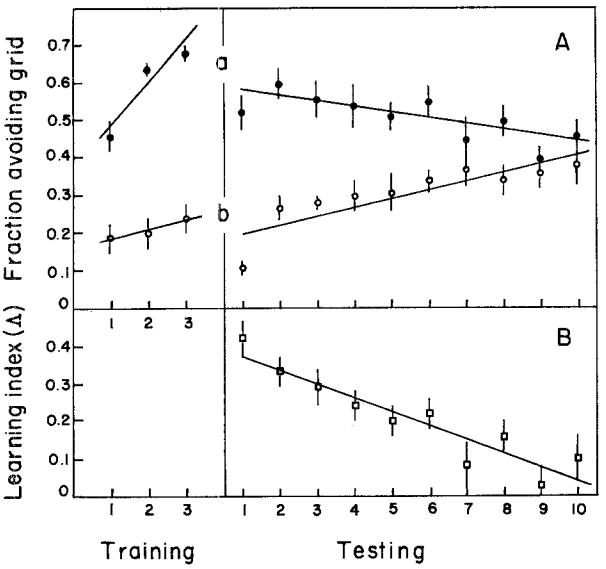

Fig. 5A and B. Extinction of the selective avoidance behavior of C-S flies during repeated testing without shock, following training in the standard paradigm. **A** avoidance of shock-associated (*a*) and control (*b*) compounds. **B** The learning index calculated from the experiments described in A. Each point represents 8 experiments

in A is due not only to a slow decrease in the avoidance of the shock-associated compound, but also to an increase in the avoidance of the control odorant (Fig. 5A, B). Such an increase in avoidance is seen also when naive flies are repeatedly run into the test tubes, even when they are run into a new grid each time. The extinction of the selective avoidance behavior displayed by trained flies (Fig. 5B) is thus superimposed on an increase in avoidance toward both the shock-associated and control odorants. The causes of the latter were discussed in Section A-2.

2. Memory Tested Without Extinction

Even after a single training cycle, selective avoidance behavior can be detected for more than an hour if not extinguished (Fig. 6b). Memory is better maintained in flies which are trained for longer periods, e.g., in a 3-training-cycle paradigm (Fig. 6a). If the standard training paradigm is repeated four times at 2 h intervals, selective avoidance ($A \approx 0.1$) is demonstrable even 24 h after the last training session (Quinn et al., 1974). In all cases, the memory decays more rapidly in the first minutes and slower later.

As is seen from Figure 6a, 1 h after a standard 3-cycle training is completed, flies retain about 50% of their original learning score. A question that can be asked is whether the saved information can be used in order to improve the learning index, i.e., whether flies which are trained (but not tested), then retrained 1 h later and tested immediately, would display a higher learning

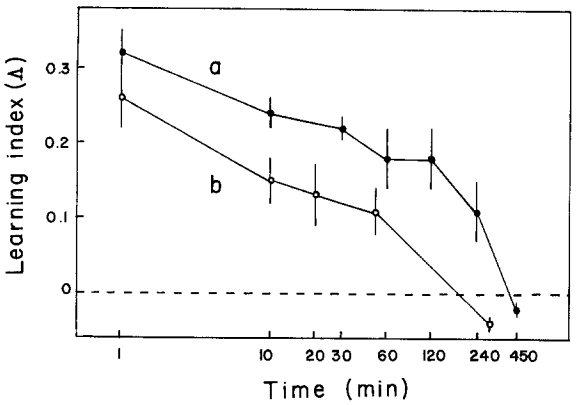

Fig. 6. Decay of memory of C-S flies with time. *a*, Following 3-training cycles. *b*, Following a single training cycle. Each point represents 4 to 8 experiments

index than those which are trained only once. C-S flies were thus trained, and retrained in a new instrument 1 h later. Indeed, on their first run into the shock-associated grid during retraining, the flies displayed higher avoidance of the odorant than they had in their first training session ($69 \pm 2\%$ avoiding in the first retraining cycle vs. $57 \pm 2\%$ avoiding in the first training cycle, 8 measurements each). However, the A after retraining was found to be 0.39 ± 0.03 (4 exps.), not significantly different from the A of C-S flies trained only once in the standard paradigm (0.34 ± 0.02, 9 exps.). The results therefore show that the stored information does not substantially increase the learning performance of flies when retrained.

3. Memory Disruption and Memory Phases

In several memory tasks it is possible to distract the organism's attention immediately after training and thus disrupt memory (Brown, 1958). Experiments were performed to test whether the same is true for flies. Flies were trained in the single training cycle paradigm and immediately transferred to a new countercurrent apparatus, in which they were banged intensely every 45 s, and between bangings allowed to geotact in the vertically held apparatus. After 10 min the flies were tested for memory. A $A = 0.15 \pm 0.02$ ($n = 4$) was obtained, the same as for control flies which were allowed to rest between training and testing. It was therefore concluded that rest following training is not necessary for normal memory.

A common method of disrupting memory in various organisms is to use pharmacological treatments (Glassman, 1969). Studies employing such agents also indicate that memory passes through two phases (Barondes, 1970; McGaugh and Herz, 1972): Short-term memory, which is maintained during and shortly after learning, and long-term memory, which is consolidated minutes after learn-

ing (McGaugh and Herz, 1972). Several agents can prevent consolidation but have little effect once it has been completed. These agents include anesthesia, e.g., by ether, CO_2, N_2 or cold, and drugs, e.g., protein synthesis inhibitors (Glassman, 1969).

The susceptibility of the fly memory to such treatments was studied. The agents tested were N_2-narcosis and cold-narcosis induced after the end of training, and cycloheximide feeding (5 mg/ml in 2% sucrose for 46 h prior to training). The flies were trained in the standard 3-training-cycle paradigm, and were tested 1 h later, as described under Methods. In control experiments it was found that flies which had been fed with cycloheximide as above, or anesthetized with N_2 or cold for 5 min, 50 min prior to training, learned normally when tested immediately.

Cycloheximide, when fed at a concentration of 5 mg/ml for more than 24 h, decreases the protein synthesis level in *Drosophila* to about 10% of its normal level (Dingley and Maynard-Smith, 1968). Such treatment had no effect on memory; A after 1 h was 0.21 ± 0.07 ($n=6$), in comparison to $A=0.18 \pm 0.04$ ($n=12$) for control.

Both cold-narcosis and N_2-narcosis abolished the memory when induced for 5 min starting 5 min after the end of training. The A obtained were 0.04 ± 0.04 ($n=8$) and 0.02 ± 0.03 ($n=6$) respectively.

As the recovery of the flies from cold-narcosis is very rapid (they behave normally about 10 min after removal from the ice bucket), this treatment seemed to be most useful for further assessing the effects of narcosis induced at different times after training. Results of such experiments are presented in Figure 7. It is seen that cold-narcosis for 5 min, starting as late as 15 min after training, abolished memory, but the same treatment starting 30 min after training had no effect. The transition between cold-sensitive and cold-insensitive phases was found to be steep, with the critical period being between 20–30 min after training. No evidence could be found in these experiments for a "gradient" of consolidation as is the case in other organisms (McGaugh and Herz, 1972). Evidence for a cold-sensitive memory storage component has been obtained independently by Quinn (Quinn and Dudai, 1976), employing a different paradigm in which the flies are presented during training with the shock-associated compound only and are cold anesthetized briefly (for 1 min) at various times afterwards. Employing the latter procedure, a consolidation gradient was observed, with cold sensitivity decreasing gradually between 0 and 30 min after training. Thus, the kinetics of consolidation seem to depend on the paradigm used. Nevertheless, both paradigms indicate that flies display two memory phases—an early one sensitive to cold-narcosis and a later one which is not sensitive to such treatment.

In contrast with cold-narcosis, the recovery of flies from N_2-narcosis is quite slow. Flies which had been anesthetized with N_2 for 5 min starting 30 min after training, remembered better than flies which had been anesthetized with N_2 for 5 min, starting 5 min after training (for tests performed 1 h after training, $A=0.09 \pm 0.01$ vs. 0.02 ± 0.03, $P<0.05$). However, the former ones displayed poor phototaxis (nonphototaxis index$=0.35 \pm 0.03$, compared to 0.07 ± 0.01 for flies tested 25 min after 5 min of cold-narcosis). The poor phototaxis probably distorted the results of the memory test.

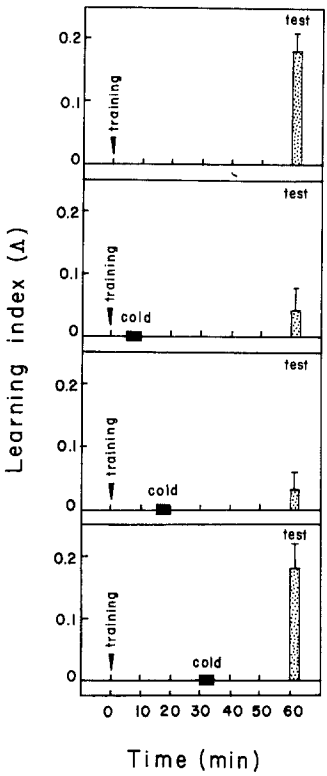

Fig. 7. Susceptibility of *Drosophila* memory to cold narcosis as a function of the time of treatment following training in a standard 3-training cycle paradigm. 6 to 12 experiments each

C. Learning Capabilities of Various Strains and Mutants

The learning properties of a sample of 44 *Drosophila* strains and morphological and behavioral mutants were studied (Table 2). Some other mutants which were found to be completely non-phototactic were not used. As can be seen from the table, there are significant differences in performance by various strains and mutants.

There are several possible reasons for poor performance in the learning paradigm used: (a) "stupidity", or inability to associate shock with odorant; (b) inability to sense the compound (probably by smell, see below) or to distinguish between the shock-associated and control compounds; (c) hyposensitivity to shock; (d) hypersensitivity to shock, causing the flies to pass out or be damaged; (e) sluggishness and/or poor phototaxis, which lead to a lack of drive toward the grids. The above factors can be distinguished by analyzing the data as in Table 2.

"Stupid" flies should fail to avoid selectively the shock-associated odorant during testing. Ideally, they should otherwise be normal with regard to their sensory and motor capabilities. None of the mutants in Table 2 seems to fit into this category. It is still possible that the molecular machinery necessary for normal learning and memory is defective in some of the mutants described

Table 2. Learning ability of various strains and mutants of *Drosophila melanogaster*. Each strain or mutant was tested 3–5 times in the standard paradigm using 0.5% 3-octanol and 0.5% 4-methylcyclohexanol as odorants

Strain/Mutant	Phenotype	Learning Index (A)[a]	Nonphototaxis Index[b]	Avoidance Index[c]	Control-Avoidance Index[d]
A=0.0–0.1					
ro/ro	Eyes rough, irregular facets[e]	0.02±0.01	0.29±0.03	0.89±0.02	0.75±0.06
gdh^{n4}/gdh^{n4}	Glutamic dehydrogenase deficient. Slow and weak	0.07±0.01	0.24±0.03	0.64±0.02	0.53±0.04
A=0.1–0.2					
PC75/PC75	Easily shocked[f]	0.11±0.04	0.60±0.05	0.71±0.05	0.71±0.03
Song mutant	Frequent and continuous buzzing of wings (Love-song mutant)[p]	0.11±0.05	0.48±0.05	0.83±0.03	0.63±0.07
Lausanne-S	**Wild-type**; slow	0.11±0.03	0.22±0.03	0.77±0.06	0.42±0.07
e^4/e^4	*ebony*; body color black. Abnormal electroretinogram. Amount of dopamine twice as normal[g]	0.12±0.04	0.02±0.01	0.57±0.03	0.33±0.05
sc/sc	Marked reduction in number of bristles. Low avoidance	0.12±0.03	0.15±0.02	0.44±0.03	0.26±0.04
KO120♂♂	Shaker under ether[h]	0.13±0.03	0.13±0.02	0.68±0.01	0.37±0.04
spl/spl	Eyes rough and small, some bristles doubled or missing	0.13±0.01	0.23±0.04	0.74±0.01	0.46±0.04
e^s/e^s	*ebony-sooty*; color lighter than *ebony* (see *ebony*, above)	0.14±0.03	0.09±0.02	0.68±0.04	0.31±0.04
adh^{n-1}/adh^{n-1}	No alcohol dehydrogenase	0.14±0.03	0.13±0.03	0.62±0.02	0.25±0.02
acph °/acph°	No alkaline phosphatase. Phototaxis variable	0.14±0.06	0.19±0.02	0.72±0.04	0.50±0.04
fruity♂♂	Abnormal courtship, males court males persistently[i]	0.14±0.07	0.41±0.04	0.81±0.03	0.46±0.05
b/b	Black	0.14±0.02	0.18±0.04	0.70±0.04	0.43±0.06

[a] Fraction of flies avoiding the shock-associated compound minus the fraction avoiding the control compound during the test. For all definitions see also under Methods
[b] Fraction of flies not entering the rest tube (i.e., not phototacting) during the test
[c] Fraction of flies avoiding the shock-associated compound in the last training cycle
[d] Fraction of flies avoiding the control compound in the last training cycle
[e] Lindsley and Grell, 1968; this reference applies to all other mutations unless otherwise indicated
[f] Benzer, 1973
[g] Hodgetts and Konopka, 1973
[h] Benzer, unpublished
[i] Gill, 1963
[j] Konopka, 1972
[k] Kaplan and Trout, 1969
[l] Hall and Kankel, 1976; Dudai, 1976
[m] Kindly provided by T. Wright, University of Virginia, Charlottesville
[n] Harris et al., 1976
[o] Sparrow and Wright, 1974
[p] Kindly provided by F. von Schilcher

Table 2 (continued)

Strain/Mutant	Phenotype	Learning Index (Λ)[a]	Nonphototaxis Index[b]	Avoidance Index[c]	Control-Avoidance Index[d]
oc ptg^3♂♂	Ocelliless, and pentagon (=thoracic parts darker)	0.15±0.03	0.23±0.03	0.90±0.03	0.60±0.09
BS64♂♂	Hyperkinetic (jumps in response to hand waving)[h]	0.16±0.06	0.26±0.04	0.79±0.05	0.44±0.07
t^3/t^3	Body color tan. Abnormal electroretinogram. Low dopamine level	0.17±0.08	0.12±0.04	0.76±0.05	0.37±0.05
v/v	Eye color vermilion	0.17±0.02	0.08±0.01	0.72±0.04	0.37±0.05
RH26♂♂	Hyperactive[h]	0.17±0.04	0.06±0.01	0.76±0.04	0.42±0.04
B/B	*Bar*: eyes narrow and small	0.17±0.06	0.20±0.03	0.79±0.04	0.57±0.05
tyr-l(p^p)	Tyrosinase deficient	0.18±0.03	0.22±0.01	0.67±0.05	0.20±0.05
Hk^1/Hk^1	Hyperkinetic[k]	0.19±0.03	0.13±0.02	0.71±0.02	0.40±0.04
Ore-R	**Wild-type**	0.19±0.02	0.32±0.03	0.83±0.02	0.49±0.05
Λ=0.02–0.3					
Swedish-C	**Wild-type**	0.20±0.01	0.16±0.03	0.61±0.03	0.35±0.05
y sn^3 car	Three mutations: yellow body; bristles twisted and shortended; eye color dark ruby	0.21±0.04	0.12±0.03	0.76±0.03	0.32±0.08
Hikone A-S	**Wild-type**	0.22±0.06	0.05±0.02	0.59±0.06	0.08±0.03
y/y	Yellow body	0.23±0.05	0.06±0.02	0.69±0.03	0.29±0.03
fruity/fruity♀♀	See fruity above; females do not court females[i]	0.23±0.04	0.14±0.05	0.57±0.03	0.28±0.06
126d/+	Heterozygous for acetylcholinesterase deficiency. Contains 50% of normal activity[l]	0.23±0.02	0.12±0.01	0.63±0.02	0.20±0.03
Sh^5Sh^5	Shaker under ether[k]	0.24±0.02	0.11±0.02	0.75±0.02	0.34±0.03
ey^2/ey^2	Eyes much smaller than normal, cephalic complex reduced	0.25±0.08	0.08±0.02	0.83±0.01	0.40±0.06
Urbana-S	**Wild-type**	0.26±0.07	0.07±0.01	0.55±0.03	0.17±0.03
f/f	Bristles shortened, bent and split	0.27±0.03	0.21±0.04	0.81±0.03	0.37±0.06
sn^3/sn^3	Bristles twisted and shortened	0.27±0.02	0.12±0.02	0.73±0.04	0.42±0.04
red/red	Malpighian tubes red, eyes brown	0.29±0.06	0.11±0.02	0.68±0.02	0.22±0.04
KS82♂♂	Hyperkinetic[h]	0.29±0.06	0.06±0.01	0.76±0.04	0.37±0.05
Λ=0.3–0.4					
1(2)Ddc^{n1}/+	Dopa-dexarboxylase deficiency[m]	0.30±0.05	0.04±0.01	0.66±0.02	0.23±0.05
KS222/KS222	Receptor cells 1–6 in eye degenerate in light[n]. Tested before degeneration	0.30±0.03	0.03±0.01	0.69±0.04	0.22±0.05
C-S	**Wild-type**	0.31±0.02	0.02±0.01	0.63±0.01	0.24±0.01
PC5/PC5	Hyperactive, shaker under ether[h]	0.32±0.06	0.11±0.02	0.73±0.02	0.28±0.03

Table 2 (continued)

Strain/Mutant	Phenotype	Learning Index (Λ)[a]	Nonphototaxis Index[b]	Avoidance Index[c]	Control-Avoidance Index[d]
LY3♂♂	Hyperactive; rhabdomere No. 7 in the eye absent[n]	0.32 ± 0.01	0.05 ± 0.01	0.66 ± 0.04	0.33 ± 0.02
$1(2)Ddc^{n2}/+$	Dopa-decarboxylase deficiency[n]	0.34 ± 0.03	0.03 ± 0.01	0.66 ± 0.04	0.20 ± 0.02
C29Hlcnbw/+	Hyperactive; hypersensitive to α-methyl-Dopa; includes mutations for eye color[o]	0.34 ± 0.09	0.04 ± 0.01	0.65 ± 0.05	0.19 ± 0.02
Eag/Eag	Shakes under ether[k]	0.38 ± 0.02	0.04 ± 0.01	0.70 ± 0.03	0.15 ± 0.02

here, but if so, the defects also affect other aspects of behavior aside from learning (see Discussion).

Inability to detect or distinguish between the odorants might be expected to result in essentially equal avoidance of both shock-associated and control compounds. Such behavior might also be expected to result from general weakness. In the latter case, however, phototaxis into the rest tube should be poor, or diminish with training (e.g., gdh^{n4}/gdh^{n4}; see below).

Hyposensitivity to shock should lead to a low avoidance index. This is displayed by the mutant sc/sc (avoids the shock-associated grid less than C-S, $P < 0.01$). Hypersensitivity to shock leading to damage would produce a high nonphototaxis index and a high control-avoidance index. This trait is displayed by the "easily-shocked" mutant PC75/PC75, which passes out when shocked.

General sluggishness or poor phototaxis should decrease the proportion of flies reaching the grids and therefore decrease the opportunity to be conditioned. Furthermore, the proportion of flies reaching the grids during testing would also be decreased. Indeed it is possible to calculate a corrected Λ by subtracting from the total number of flies those which are not phototactic during the test. Such a calculation increases the "Λ" of some strains, but also introduces more variance into the results, as the number of flies taken into account is smaller. For example, for the song mutant, a corrected Λ of 0.19 ± 0.10 is obtained (instead of 0.11 ± 0.05, see Table 2); for fruity♂♂, the Λ becomes 0.30 ± 0.18 (0.14 ± 0.07 in Table 2); for spl/spl, the Λ becomes 0.27 ± 0.03 (instead of 0.18 ± 0.03). For other strains, the "corrected Λ" thus calculated remains small (ro/ro, 0.08 ± 0.05; gdh^{n4}/gdh^{n4}, 0.10 ± 0.02). It should be added that there are cases in which the flies display poor phototaxis during the first training cycles, but improve their phototaxis afterwards (e.g., t^3/t^3). For the latter the nonphototaxis index, which is calculated on the basis of the behavior during the test, is not an appropriate measure of the behavior during training.

Flies with a nonphototaxis index of 0.15 and higher usually perform poorly in the paradigm ($\Lambda < 0.2$). From the data presented in Table 2, a correlation coefficient, $r = -0.6$, was calculated between the nonphototaxis index and the learning index. Among the exceptions is the mutant *ebony*, in which phototaxis

is strong but the performance in the paradigm is quite poor. The learning index of *ebony* is low because during the test the flies avoided to a high and similar degree both the shock-associated and the control compounds.

Among the wild-type strains tested, C-S was found to learn the best. This strain was originally chosen by Benzer (1967) for best phototaxis. C-S flies are therefore best suited for learning mutant screening (see Discussion).

Discussion

From observing the flies inside the training tube it can be estimated that, on the average, a fly is shocked for not more than 2 to 3 s if it steps on the shock grid. Such a brief exposure to a shock-associated compound appears to be sufficient to induce conditioned behavior in *Drosophila.* However, from the findings described in the Results it is clear that the behavior of the flies in the paradigm is not dictated solely by conditioning via the electric shock.

After a single presentation with the shock-associated odor, more than half the flies avoid the odorant when presented on a new grid (without shock) within 2 to 3 min. The avoidance increases up to about 70% when the exposure to the shock-associated compound is repeated. But the avoidance of the control compound during testing reduces the learning index to about half the value of the absolute avoidance of the shock-associated compound. There are several possible causes for the control-avoidance. Some of the compounds used as cues are slightly repellent to the flies. However, the avoidance increases when the flies are repeatedly run into the control compound and seems therefore to be partially acquired. This acquisition may be the result either of sensitization or of negative reinforcement inherent in the procedure employed to evoke phototaxis and to transfer flies from tube to tube. Such an increase is also observed when flies are repeatedly run into an odorant without any shock, and therefore is not a result of incomplete dissociation of the shock from the non-shock compound.

Thus, although an apparent $A > 0.5$ can be routinely obtained by omitting the control odor during training, part of the A so obtained might not be directly attributed to the defined negative reinforcement, i.e., the electric shock, and only A values obtained from the "standard" paradigm can be regarded as measures of selective avoidance resulting from associating compound with shock. The $A \approx 0.4$ obtained in the "standard" paradigm appears to be a saturation value for the fly performance under such circumstances. Several findings are consistent with the latter assumption: a similar A is obtained in the standard paradigm using different compounds belonging to different chemical classes in different combinations; the A cannot be significantly increased by further training or by superimposing stored information on newly learned information; the A is a homogeneous property of the population (Quinn et al., 1974); and the same value is also obtained by averaging the results of experiments done with single flies, under which conditions stampede effects are eliminated (Byers, unpublished). Nevertheless, from what has been said above one should not conclude that the fly learning process is limited to a success probability of ≈ 0.4; this value could merely reflect the limitations of the paradigms used.

Memory experiments indicate that, in analogy with other organisms (Barondes, 1970; McGaugh and Herz, 1972), including insects (Erber, 1975), *Drosophila* display two memory phases, as functionally defined by the sensitivity to narcosis. However, additional experiments are needed to further characterize the phenomenon in *Drosophila* (e.g., its dependence on the paradigm used).

The degree of protein synthesis inhibition caused by cycloheximide treatment, i.e., about 90% (Dingley and Maynard-Smith, 1968) is similar to that reported for other organisms in which such treatment did abolish consolidation of long-term memory (Barondes and Cohen, 1968). Dingley and Maynard-Smith (1968) ruled out the possibility that in *Drosophila* there are protected organs in which preferential protein synthesis continues following cycloheximide feeding. It is possible, therefore, that protein synthesis is not necessary for "consolidation" in *Drosophila*. However, an alternative explanation is that sometimes even 90% of protein synthesis may not be sufficient for interference with the establishment of long-term memory (Barondes and Cohen, 1967). Increasing cycloheximide doses does not increase protein synthesis inhibition in *Drosophila* more than $\approx 90\%$ (Dingley and Maynard-Smith, 1968).

In the experiments described it is likely that the olfactory sense, rather than taste, plays the major role in mediating the sensory cue during conditioning. Substances which are detected by the flies while in solution, but which have low vapor pressures (e.g., sucrose and fructose), do not work well when applied to the grid. In addition, conditioned flies are often seen turning away from the grid tube during the test even before entering it and having an opportunity to taste it. However, a role of the gustatory sense cannot be completely ruled out. In any case, it is not only the pure chemical to which the flies are exposed. The compound is spread on a metal and plastic grid, which has a distinguishable odor of its own, and which the flies can learn to avoid even without any additional odor. The flies themselves change the odor of the grid during repetitive runs into it, especially if they are shocked on the same grid.

It is not known to what extent fly learning plays a significant role in nature. An ability to learn to avoid poisonous food is a potential advantage, but there is no evidence that during its lifetime the fly makes use of this potential. It is also possible that an ability to learn is a basic property of any complex nervous system, even in an organism in which most of the normal behavioral repertoire is dictated by inborn behaviors. In any case, the learning and memory processes in *Drosophila* seem to be quite complex (e.g., including the ability to acquire and store multiple items of information concomitantly), and many of their properties are analogous to those displayed by other organisms. The use of this system for studying the genetic basis of learning and memory seems therefore appropriate. Its main advantage is the relative ease by which single gene mutants can be isolated and genetically characterized.

Mutants screened for their learning and memory ability can either be the progeny of mutagen-treated flies (Benzer, 1967), or existing morphological (Lindsley and Grell, 1968) and behavioral (Benzer, 1973) mutants. This is the first time that the effects of many independent single-gene mutations on the learning ability of an organism have been tested (Table 2). The mutants include some with lesions in different components of the nervous system (e.g., sc, ey,

hyperkinetics, shakers), some with deficiencies in enzymes known to be involved in nervous activity (e.g., acetylcholinesterase, catecholamine metabolism), and others. A comparison of the learning performance of various wild-type strains and morphological and behavioral mutants reveals differences among them. Of the mutants described in this paper none can be classified as simply "stupid", because defects in performance might be attributed to differences in various aspects of behavior (e.g., general activity, phototaxis, shock sensitivity). However, it is still possible that some of the mutations tested interfere with the molecular machinery needed for learning and memory, or with accessory processes necessary for learning and memory, but that these mutations also affect other behaviors. For example, a positive correlation was found above between phototaxis and learning ability. Rapid phototaxis is initiated and maintained by excitation and arousal and defects in such processes may also interfere with information storage and retrieval (Kety, 1970). Because the paradigm requires a substantial level of arousal for proper sensory and locomotor responses, it cannot be used as it is for further studies of the relationship between mutations causing arousal defects and learning.

Especially relevant to the subject of mutant screening is the finding that various *Drosophila* wild-type strains differ in their ability to display learning in the paradigm. A difference in behavioral performance resulting form different genetic backgrounds of existing mutants might therefore lead to differences in the learning performance which are not related to the mutation tested, nor even to learning mechanism per se. In trying to locate genetic lesions which specifically affect learning and memory, it is therefore preferable to look for single-gene mutations on a uniform genetic background (Benzer, 1967). Among the wild-type strains tested, C-S flies seem to be the best candidate for mutagenesis. The isolation of an X-chromosome mutant deficient in learning, found among the progeny of ethylmethanesulfonate treated C-S flies, is reported elsewhere (Dudai et al., 1976).

I thank Dr. S. Benzer for the hospitality of his laboratory, and S. Benzer, D. Byers, W. Harris, L. Jan, Y.-N. Jan, W. Quinn, D. Ready and M. Shankland for valuable discussions. This work was supported by an EMBO long-term fellowship and by a grant from the National Science Foundation to Dr. S. Benzer.

References

Bailey, N.T.J.: Statistical methods in biology. London: The English University Press 1968

Barondes, S.H.: Multiple steps in the biology of memory, pp. 272–278. In: The neurosciences: Second research program (F.O. Schmitt, ed.). New York: The Rockefeller University Press 1970

Barondes, S.H., Cohen, H.D.: Delayed and sustained effect of acetoxycycloheximide on memory in mice. Proc. nat. Acad. Sci. (Wash.) **58**, 157–164 (1967)

Barondes, S.H., Cohen, H.D.: Arousal and the conversion of "short-term" to "long-term" memory. Proc. nat. Acad. Sci. (Wash.) **61**, 923–929 (1968)

Benzer, S.: Behavioral mutants of *Drosophila* isolated by countercurrent distribution. Proc. nat. Acad. Sci. (Wash.) **58**, 1112–1119 (1967)

Benzer, S.: Genetic dissection of behavior. Sci. Amer. **229**, 24–37 (1973)

Brown, J.A.: Some tests of the decay theory of immediate memory. Quart. J. exp. Psychol. **10**, 12–21 (1958)

Byers, D., Quinn, W.G.: Experiments on learning in *Drosophila*: effects of anesthesia and screening for learning mutants, p. 105. In: Caltech 1974 Biology Annual Report. Pasadena: California Institute of Technology 1974

Dethier, V.-G.: Insects and the concept of motivation, pp. 105–136. In: Nebraska Symposium on motivation, Vol. 14 (D. Levine, ed.). Lincoln: University of Nebraska Press 1966

Dingley, F., Maynard-Smith, J.: Temperature acclimatization in the absence of protein synthesis in *Drosophila subobscura*. J. Insect Physiol. **14**, 1185–1194 (1968)

Dudai, Y.: Molecular states of acetylcholinesterase from *Drosophila melanogaster*. Dros. Inf. Serv. **52**, in press (1976)

Dudai, Y., Jan, Y.-N., Byers, D., Quinn, W.G., Benzer, S.: *dunce*, a mutant of *Drosophila* deficient in learning. Proc. nat. Acad. Sci. (Wash.) **73**, 1684–1688 (1976)

Erber, J.: The dynamics of learning in the honey bee (*Apis mellifica carnica*): II. Principles of information processing. J. comp. Physiol. **99**, 243–255 (1975)

Gill, K.S.: A mutation causing abnormal mating behavior. Dros. Inf. Serv. **38**, 33 (1963)

Glassman, E.: The biochemistry of learning: an evaluation of the role of RNA and protein. Ann. Rev. Biochem. **38**, 605–646 (1969)

Hall, J.C., Kankel, D.R.: Gene controlling acetylcholinesterase in *Drosophila melanogaster*. Genetics **83**, 517–535 (1976)

Harris, W.A., Stark, W.S., Walker, J.A.: Genetic dissection of the photoreceptor system in the compound eye of *Drosophila melanogaster*. J. Physiol. (Lond.) **256**, 415–439 (1976)

Hodgetts, R.B., Konopka, R.J.: Tyrosine and catecholamine metabolism in wild-type *Drosophila melanogaster* and a mutant, *ebony*. J. Insect Physiol. **19**, 1211–1220 (1973)

Kaplan, W.D., Trout, W.E.: The behavior of four neurological mutants of *Drosophila*. Genetics **61**, 299–309 (1969)

Kety, S.S.: The biogenic amines in the central nervous system: their possible roles in arousal, emotion and learning, pp. 324–336. In: The neurosciences. Second study program (F.O. Schmidt, ed.). New York: The Rockefeller University Press 1970

Konopka, R.J.: Abnormal concentrations of dopamine in a *Drosophila* mutant. Nature (Lond.) **239**, 281–282 (1972)

Lewis, E.B.: A new standard food medium. Dros. Inf. Serv. **34**, 117–119 (1960)

Lindsley, D.L., Grell, E.H.: Genetic variation of *Drosophila melanogaster*. Carnegie Institution of Washington, Publ. No. 627, Washington, D.C. (1968)

Manning, A.: "Pre-imaginal conditioning" in *Drosophila*. Nature (Lond.) **216**, 338–340 (1967)

McGaugh, J.L., Herz, M.-J.: Memory consolidation. San Francisco: Albion Publishing 1972

Murphy, R.M.: Spatial discrimination performance of *Drosophila melanogaster*: test-retest assessments and reinterpretation. Anim. Behav. **21**, 687–690 (1973)

Nelson, M.C.: Classical conditioning in the blowfly (*Phormia regina*): associative and excitatory factors. J. comp. physiol. Psychol. **77**, 353–368 (1971)

Quinn, W.G., Dudai, Y.: Memory phases in *Drosophila*. Nature (Lond.) **262**, 576–577 (1976)

Quinn, W.G., Harris, W.A., Benzer, S.: Conditioned behavior in *Drosophila melanogaster*. Proc. nat. Acad. Sci. (Wash.) **71**, 708–712 (1974)

Sparrow, J.C., Wright, T.R.F.: The selection for mutants in *Drosophila melanogaster* hypersensitive to a-methyl DOPA, a DOPA-decarboxylase inhibitor. Molec. gen. Genet. **130**, 127–141 (1974)

Spatz, H.Ch., Emanns, A., Reichert, H.: Associative learning of *Drosophila melanogaster*. Nature (Lond.) **248**, 359–361 (1974)

Yetmann, F.R., Hirsch, J.: Attempted replication of, and selective breeding for, instrumental conditioning of *Drosophila melanogaster*. Anim. Behav. **19**, 454–462 (1971)