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Drug Disruption of Short-Term Memory in *Drosophila melanogaster*

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XIA, S. Z., L. LIU, C. H. FENG AND A. K. GUO. *Drug disruption of short-term memory in* Drosophila melanogaster. PHARMACOL BIOCHEM BEHAV **58**(3) 727–735, 1997.—Recent work on operant visual learning and memory in *Drosophila* has suggested at least three distinct memory phases. Trying to disrupt memory pharmacologically, we fed flies with ouabain or the depolarizing drugs potassium chloride (KCl), lithium chloride (LiCl) and monosodium glutamate for some specific time before training. The depolarizing drugs abolished memory very soon after training. Ouabain exerted no effect on memory within the first 20 min but abolished it more than 30 min after training. These drugs had no diminishing effects on the visual discrimination and behavioral performance of the flies during training. This result suggests that memory disruption may not be induced by nonspecific effects of the drugs. In addition, reversal training of the KCl-fed flies indicates that KCl appears not to impair the retrieval mechanism of flies. These results suggest that the specific disruptive effects of the drugs on memory formation and the existence of a short-term memory phase, are susceptible to disruption of the depolarizing drugs but unaffected by ouabain. © 1997 Elsevier Science Inc.

Drosophila melanogaster Operant conditioning Visual associative learning Short-term memory Depolarizing drug Potassium chloride Lithium chloride Monosodium glutamate Ouabain

DROSOPHILA melanogaster have been introduced into the studies of learning and memory because they can learn a variety of associative tasks and are readily amenable to genetic analysis (7,21,36). Based on their flight orientation behavior, which has been proven to be operant (17,19,40,41), one novel learning paradigm has been introduced for *Drosophila* (42). This paradigm involves a visual-pattern (active) avoidance conditioning of individual tethered flies. During training, two equally attractive visual pattern types (i.e., upright T and inverted T) are used as visual landmarks on a panorama surrounding the fly; with one of the patterns in the frontal quadrant of the panorama, the animal is "punished" with heat as negative reinforcement; with the other pattern, there is no punishment. The fly is located in the center of the panorama and is able to escape the heat punishment under its own control on the flight directions with respect to landmarks. Afterward, the fly stays in the same situation, in which it makes its choice between the two patterns for testing learning acquisition or memory formation without heat reinforcement. This learning task is an operant situation in which the fly receives training and testing based on its visual recognition or discrimination (42). In addition, the behavior of individual flies is better controlled than "freely" walking or flying animals, and the whole sequence of individual performance can be measured and analyzed. The controllability and measurement of both the sensory input and the flight traces of individuals make this learning paradigm especially valuable for fine-scale and detailed investigations of the interplay between sensory and motor processes in visual associative learning.

Congruent lines of studies have suggested an intricate multiphasic pathway of memory consolidation. The multiple phases of memory emerge at different times after training, and their duration and times of onset can vary with different tasks and species (3,8,26,31). One common behavioral feature is that memory is consolidated into a longer-lasting stable form from a short-living labile form that exists immediately after training. During this consolidation period, memory can be disrupted or blocked by administration of anesthetics or protein synthesis inhibitors (3,13–15,24,26,38,39). In the present study, several drugs were employed to prevent memory formation in flies subjected to operant conditioning, including ouabain and the depolarizing drugs potassium chloride (KCl), lithium chloride (LiCl) and monosodium glutamate (13,14,39). The respective control experiments were always carried out in parallel (i.e., alternating individuals of the same population of flies) with the referring experiments.

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METHODS

Subjects

Drosophila melanogaster of the wild-type strain, Berlin were used in this study. Flies were grown at 24 ± 1°C in a 14-h: 10-h light/dark cycle, with lights on at 7 AM, and bred on standard corn meal/molasses food medium ["Wuerzburg recipe," see (71)].

Experimental flies were transferred to fresh food vials 0–24 h after hatching, where they were raised for about 36 h. Single flies were prepared with a small hook of copper wire (0.05 mm diameter) glued to the head and thorax (20). Individual flies were put into small transparent chambers, where they stayed overnight to become accustomed to the triangle hooks on the back. Experiments were carried out on single flies between 8 AM and 8 PM the following day. No sex-related differences in learning ability were apparent. However, male flies appeared to have a more persistent flight behavior. Thus, male flies were used in all experiments. Each sample point included 8–10 pairs of flies, i.e., the paired measures from 2 flies in which one fly had the upright T and the other the inverted T associated with heat as negative reinforcement.

Drugs and Feeding Regimen

The flies with the head and thorax glued together were fed with KCl (25–200 mM; Beijing, Analytical Grade), LiCl (80 mM; Beijing, Analytical Grade), monosodium glutamate (50 mM; L-glutamic acid, sodium salt, Sigma) ouabain (0.5 mM; Sigma) in 5% sugar solution (w/v) for a specific time interval, or sugar solution alone for 12 h before training as control. Flies were placed singly in small transparent chambers with a filter paper on the bottom that was soaked in one of the above solutions. About 5 min before training began, a single fly was fixed to the torque meter and allowed to groom itself on a small piece of tissue soaked with distilled water. Immediately following training, the flies were lifted out of the panorama and fed with the solution. The tissue was then exchanged with a new wet tissue soaked with distilled water, which the flies kept for various retention intervals tested.

For experiments testing for learning acquisition, the flies were fed with 5% sugar solution alone or laced with one of the solutions for more than 12 h before training, as described above, but not after training. They were tested for learning acquisition immediately after training.

Training and Testing

The learning apparatus (i.e., the flight simulator) and the conditioning procedure have been described previously (42,46,47). Briefly, the flight simulator establishes normal negative feedback between the fly's yaw torque and angular velocity of a visual panorama surrounding the animal [coupling coefficient $K = -11°(s10^{-10}\ Nm)^{-1}$; for details, see (20,42)]. One single fly is fixed to a torque meter measuring its yaw torque, placed in the center of the vertical panorama illuminated from behind and allowed to control angular velocity of the panorama with the own yaw torque in a negative feedback loop. The angular position of the panorama (i.e., the fly's flight orientation with respect to the visual landmarks at the wall of the panorama) is detected and stored continuously in a computer for the purpose of evaluating the learning scores and for control of the reinforcer. The visual landmarks consist of 4 equally sized, T-shaped black patterns; two of them (opposing quadrants) are inverted. Negative reinforcement is provided by a microscope lamp that is lit during training but not during testing. The light beam is focused onto the fly from above by a lens and can be intercepted by a computer-controlled shutter. The heat spot is about 3 mm in diameter and covers the whole abdomen of the animal.

The conditioning procedure was basically the same as that used previously (46). In brief, training and testing were performed on the fly in the following order: the pretraining session, two training sessions, and the test session. The pretraining session consisted of three consecutive 2-min test periods during which the animal flew in a closed loop without heat reinforcement to learn how to stabilize the panorama (17). At the same time, it was tested for its spontaneous preference with respect to the two visual patterns. The training session consisted of two 2-min training periods and one 2-min test period. During these training periods, the computer-controlled infrared beam, focused on the fly, was switched on whenever the fly flew toward a quadrant that contained the upright T. When the inverted T was in its frontal visual field, the beam was intercepted by the computer-controlled shutter. Following training, in a 2-min test period the fly was tested for its learning acquisition. The whole sequence (one training session) was repeated once. Finally, the fly was tested for learning acquisition or memory formation in one test session of three 1-min test periods without heat reinforcement. Each fly was tested only once at some retention interval after training to avoid "active" memory decay caused by repeated testing. Before testing, the panorama was set to a new random position. As for the first fly, it was always conditioned to avoid the upright T paired with heat. Half of the flies were trained and tested with the upright T and the other half with the inverted T as the heat-associated pattern.

Reversal Conditioning

In the experiments involving reversal training, the flies were first conditioned to avoid one of the two visual patterns associated with heat, and subsequently the contingency between heat and patterns was switched such that the flies had to avoid the previously no-heat-associated pattern [(17, cf. (37)]. In reversal experiments (Fig. 6), the whole conditioning procedure consisted of one 10-min initial training session, one 5-min rest interval, one 10-min reversal training session and one 3-min test session. Single flies were trained to avoid one visual pattern associated with heat during the first 10-min training session (initial training, IT), and then retrained 5 min later to avoid the other pattern during the second 10-min training session (reversal training, RT), i.e., if the upright T was the heat-associated pattern in the IT session, then the inverted T was the heat-associated pattern in the RT session. Learning acquisition (LA) was measured immediately after the two training sessions, and memory retention (MR) was tested 5 min after reversal training.

Evaluation of Data

The whole sequence of pattern motion for each fly was digitally recorded in a computer. Performance indices (PI; pattern preference index before, avoidance index during and learning index after training) were calculated for a flight period as $PI = (t_1 - t_2)/(t_1 + t_2)$, with t_1 and t_2 indicating the time the fly spent fixating the no-heat- and heat-associated quadrants, respectively. The pattern preference index (PPI) is defined as the maximal absolute PI ($|PI|_{max}$) of the three PIs (i.e., for three 2-min periods) during the pretraining session. The index is a measure of the fly's ability to stabilize the arena (17) and indirectly reflects the fly's visual perceptual ability and vi-

sual discrimination necessary in this learning paradigm. The learning index (LI) during one test session or the avoidance index (AI) during one training period is defined as the average of PIs of 2 flies from one paired measure to rule out any possible spontaneous pattern preference or asymmetry of the setup (47). LI is a measure for the pattern-specific avoidance behavior acquired from training; i.e., the fraction of the time for the subject to avoid the heat-associated pattern minus that for it to avoid the alternative pattern during a test session. Values of LI range from −1 to 1, with 0 indicating no learning, 1 indicating perfect learning and −1 indicating perfect "wrong decision." AI is a measure of the pattern-specific avoidance behavior shown by the fly to avoid heat punishment during training. An index value of 1 indicates complete avoidance of the heat-associated pattern and −1 indicates complete fixation of the heat-associated pattern.

Error bars in all figures indicate standard errors of the mean (SEMs). Samples (N) for experiments using LIs or AIs indicate the number of the paired measures from 2 flies (one with upright T and the other with the inverted T associated with heat); samples (n) for experiments using PPIs indicate the number of flies tested. Because PIs calculated as $(t_1 - t_2)/(t_1 + t_2)$ have been determined empirically to distribute normally (data not shown), PPIs as well as LIs and AIs as defined above also should distribute normally (33). Thus, statistical significances of the differences among two or more means of untransformed (raw) data were assessed with analysis of variances (ANOVA); if necessary, Tukey's honestly significant difference method (T-method) was used to assess unplanned pairwise comparisons between group means (33). Comparisons between the two means were also assessed with Student's t-test.

RESULTS

Normal Retention in Flies

Although we have demonstrated that memory can be retained for at least 12 h after training with the conditioning procedure used here (46), we have failed to show a reasonably full time course of normal retention. Before attempting to disrupt memory pharmacologically, different groups of flies were tested for memory at various times after training (Fig. 1A) to establish the normal retention of the learning task. Memory retention attenuated more quickly during the first 30 min, much slower in the following 30 min after training, and again quickly afterward. The acquired heat avoidance behavior was still present (0.18 ± 0.04) at 180 min after training.

To analyze memory soon after training in more detail, populations of flies were tested for the acquired heat-avoidance behavior immediately (0) or at 1, 2, 3, 5, 10, 20 after training (Fig. 1B). The value of learning acquisition (0.37 ± 0.04), was similar to that reported previously (46). A transient decrease in retention was observed within 1–3 min after training [$t(22) = 2.42, 2.06$; $p < 0.05$, $= 0.05$ for LI at 2 min vs. LIs at 0 and 3 min, respectively]. Memory retention attenuated much more slowly after 3 min.

Effect of KCl Feeding Time on Memory Formation

Populations of flies were fed 75 mM KCl (KCl+; open squares) as described in Methods for 12, 6, 2, 1, 0.5 h, just before or immediately after training and then were tested for memory retention at 10 min after training (Fig. 2A). Another group of flies was tested for 10-min memory retention when fed 5% sugar solution alone (control; closed square) for more than 12 h before training. A one-way ANOVA with group as main effect produced a significant between-group effect [$F(7, 64) = 11.3$, $p < 0.001$]. T-method ($\alpha = 0.05$) confirmed that (a) the groups fed the drug for 2, 6 or 12 h before training, which did not differ from each other, differed from the groups fed the drug for 0.5 h or just before or immediately after training and the control, which also did not differ from each other, and (b) the −1-h group differed from the control but did not differ from the rest groups. In addition, t-tests indicated that LIs of the −2-, −6- and −12-h groups were all near zero [$t(8) \leq 0.94$; $p > 0.4$], and LI of the −1-h group was greater than zero [$t(8) = 2.94$; $p < 0.02$]. The results indicate that the drug feeding more than 1 h before training disrupts memory formation in flies.

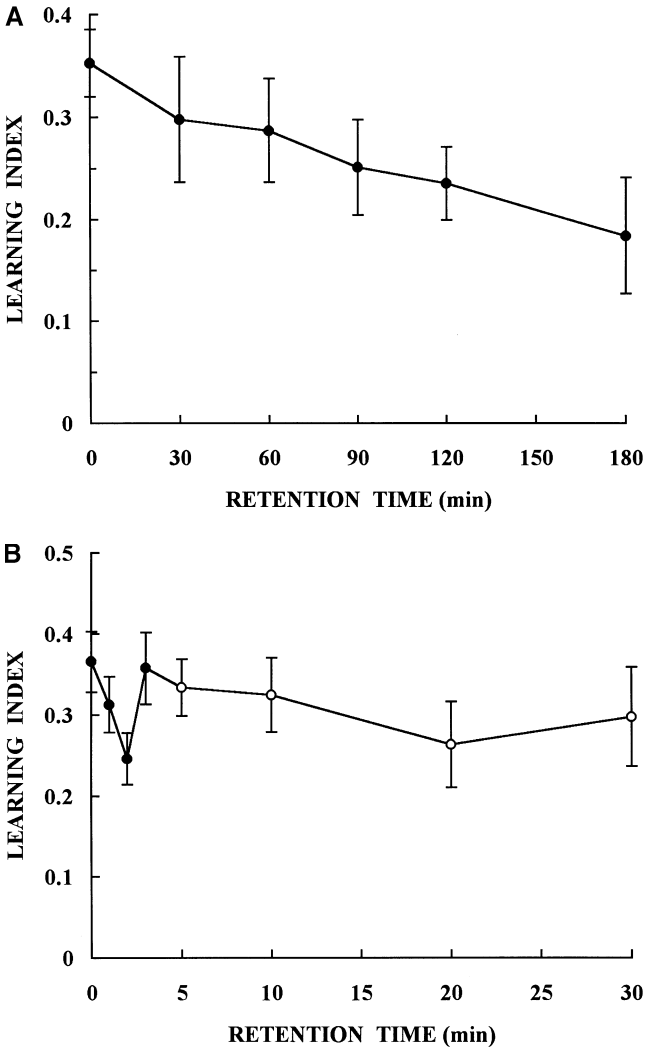

FIG. 1. Normal memory retention after operant visual learning. A: Populations of the male wild-type flies were tested for memory retention at 0, 30, 60, 90, 120, 180 min after training. B: The flies were tested for the acquired heat avoidance behavior at 0, 1, 2, 3, 5, 10, 20, 30 min after training with the test periods shortened to 0.5 min. $N = 12$ for the first four LIs [closed circles; six (3×2) flies were introduced later into each group to detect a significant between group difference]; $N = 9$ for the other groups.

Concentration Effect of KCl Feeding on Memory

Different groups of flies were tested for 10-min memory retention (Fig. 2B); flies were fed with 25, 50, 75, 100 or 200 mM KCl in 5% sugar solution for 1 (closed circles) or 12 (open circles) h before training, respectively. A two-way ANOVA, with concentration and feeding time as main effects, indicated that (a) the five concentrations produced different effects on memory [$F(4, 80) = 11.2$, $p < 0.001$], (b) the two feeding times produced different effects on memory [$F(1, 80) = 7.24$, $p < 0.001$] and (c) time and concentration did not interact [$F(4, 80) = 1.43$, $p > 0.1$]. *T*-methods ($\alpha = 0.05$) from separate one-way ANOVAs confirmed that (a) when the feeding regimen was introduced 1 h before training, memory retention was significantly reduced in 100- and 200-mM groups but unaffected in 25-, 50- and 75-mM groups; and (b) when introduced 12 h before training, memory retention was significantly reduced in all but the 25-mM group.

When experiments were performed to assess the disruptive effects of LiCl and monosodium glutamate on memory, very similar results were obtained (data not shown), and the conditioned performance of these flies paralleled that of the KCl-fed flies in most respects. Taken together, these results suggest that the employed depolarizing drugs can disrupt memory formation in flies by the present feeding regimen with the appropriate concentrations and feeding times before training.

Disruptive Curves of the Depolarizing Drugs

Different groups of flies were subjected to operant training and then tested for learning acquisition or memory retention at various intervals (Fig. 3); flies were fed with 5% sugar solution alone (closed squares) or laced with 75 mM KCl (KCl+; closed circles), 80 mM LiCl (LiCl+; open circles) or 50 mM monosodium glutamate (glutamate+; open squares) for more than 12 h before training. A two-way ANOVA, with feeding regimen and time as main effects, indicated that the four feeding regimens produced different effects on memory formation [$F(3, 140) = 4.68$, $p < 0.01$]. *T*-methods ($\alpha = 0.05$) from separate one-way ANOVAs confirmed that KCl, LiCl and glutamate all exerted a significantly diminishing effect on memory later than 5 min after training but produced no effect on learning acquisition measured immediately after training. In addition, *t*-tests indicated that KCl and LiCl abolished memory at 5 min, and no recovery of memory was detected at 90 min after training [$t(7) \leqslant 2.03$, $p > 0.05$ for all comparisons of KCl+ or LiCl+ vs. zero]. As for glutamate, although recovery of memory was observed at 60 and 90 min after training [$t(7) \geqslant 2.49$, $p < 0.05$ for glutamate+ vs. zero], memory retention was significantly lower than that of the control at all sampled points [$t(14) \geqslant 2.43$, $p < 0.05$].

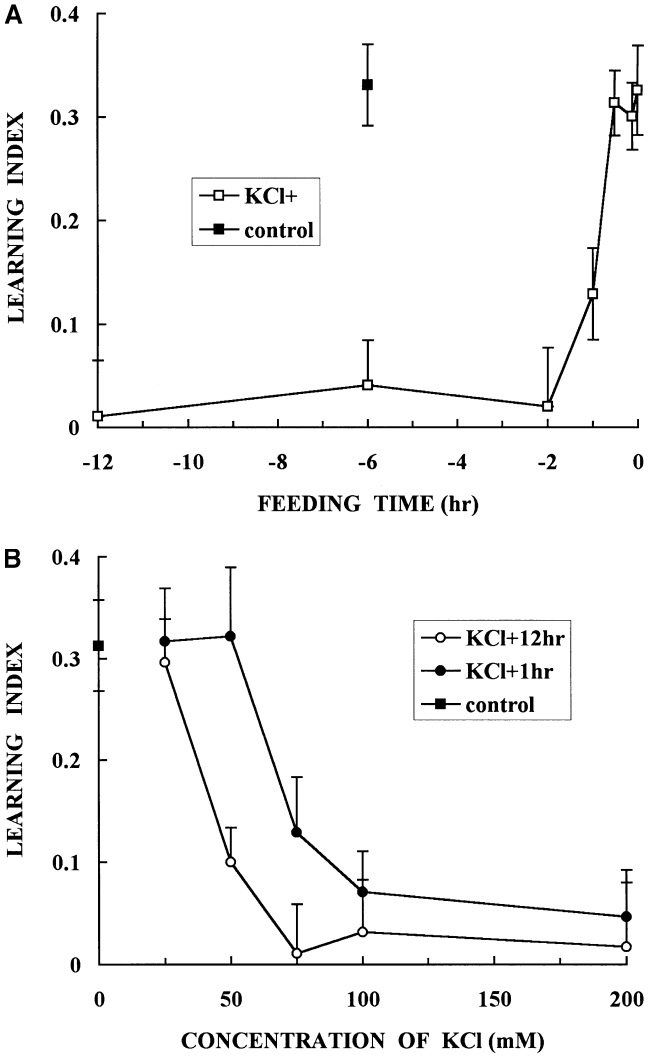

FIG. 2. Disruptive effect of KCl on memory formation. A: Different groups of flies were tested for memory retention at 10 min after training when fed 5% sugar solution alone for 12 h (closed square) or laced with 75 mM KCl (open squares) for 12, 6, 2, 1, 0.5 h, or just before or immediately after training (0 h). B: Populations of flies were measured for the 10-min memory retention after having been fed with sugar solution alone for 12 h (closed square), laced with 25, 50, 75, 100, and 200 mM KCl for 1 (closed circles) or 12 (open circles) h before training, respectively. $N = 9$ for each point.

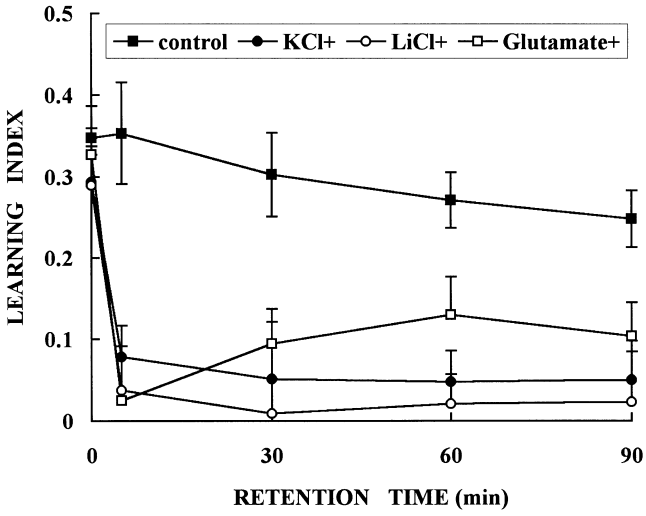

FIG. 3. Disruption of memory by the depolarizing drugs. Different groups of the flies were tested for memory retention at 0, 5, 30, 60, 90 min after training, which had been fed with 75 mM KCl (closed circles), 80 mM LiCl (open circles) or 50 mM monosodium glutamate (open squares) in 5% sugar solution or sugar solution alone (closed squares) for more than 12 h before training. $N = 8$ for each point.

Different Disruptive Effects of KCl and Ouabain

The flies, fed with 5% sugar solution alone (closed squares) or laced with 75 mM KCl (KCl+; closed circles), or 0.5 mM ouabain (open circles) for 12 h before training were tested for memory retention at 0, 10, 20, 30, 60, and 180 min after training (Fig. 4). A two-way ANOVA, with feeding regimen and time as main effects, indicated that the three feeding regimens produced different effects on memory formation [$F(2, 126) = 28.8$, $p < 0.001$]. *T*-methods ($\alpha = 0.05$) from separate one-way ANOVAs confirmed that (a) the three regimens produced no different effect on learning acquisition, (b) KCl produced LIs significantly lower than ouabain and the control starting after 10 min and (c) ouabain produced LIs significantly lower than the control starting after 30 min following training. In addition, *t*-tests indicated that ouabain abolished memory later than 30 min after training [$t(7) \leqslant 1.89$, $p \geqslant 0.1$ for ouabain+ vs. zero]. The results suggest that the depolarizing drugs such as KCl and ouabain may disrupt the formation of different memory phases in flies.

Spontaneous Pattern Preference, Avoidance Performance and Learning Acquisition Unaffected by Drug Feeding

PPIs and AIs of the flies are shown in Fig. 5. These flies were fed with 75 mM KCl (KCl+; stippled columns), 80 mM LiCl (LiCl+; stripped columns), 50 mM monosodium glutamate (Glutamate+; gray columns) or 0.5 mM ouabain (ouabain+; white columns) in 5% sugar solution or sugar solution alone (crossed columns) for 12 h before training. A one-way ANOVA revealed no significant difference between groups [$F(4, 95) = 0.16$, $p > 0.2$] for PPIs of the flies (Fig. 5A). A two-way ANOVA, with feeding regimen and training period as main effects, indicated that the five feeding regimens produced no different effect on AIs [Fig. 5B; $F(4, 149) = 0.19$, $p > 0.2$] and that the four training periods produced different effects on AIs [$F(3, 140) = 7.1$, $p < 0.001$], indicating that these flies improved their performance continuously as training proceeded.

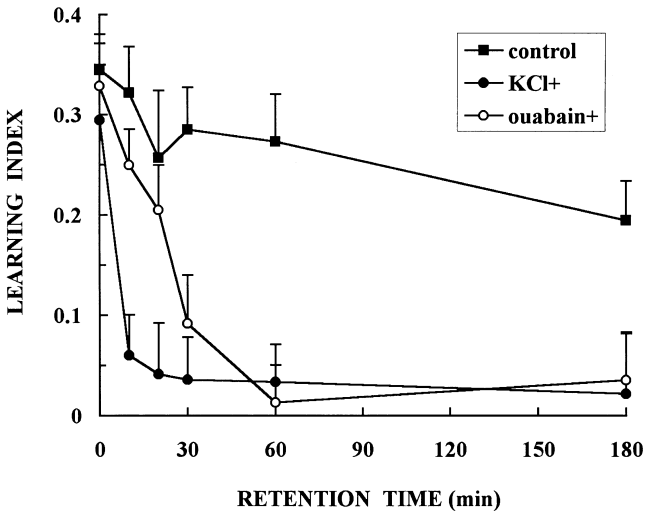

FIG. 4. Memory disruption by applying KCl- or ouabain-feeding regimens. Different groups of flies, fed with 5% sugar solution alone (closed squares), or laced with 75 mM KCl (closed circles), or 0.5 mM ouabain (open circles) for more than 12 h before training respectively, were tested for memory at 0, 10, 20, 30, 60, 180 min after training. $N = 8$ for each point.

Learning acquisition, shown as LIs at 0 min in Figs. 3 and 4, also was unaffected by these feeding regimens. When compared with the control, KCl+, LiCl+, and glutamate+ groups all produced normal learning acquisition [Fig. 3; $t(14) \leqslant 1.05$, $p > 0.3$]. Similarly, ouabain produced no effect on learning acquisition in flies [Fig. 4; $t(14) = 0.31$, $p > 0.7$ for ouabain+ vs. control].

Reversal Training of the KCl-Fed Flies

Different groups of flies were subjected to reversal training (Fig. 6); flies were fed 75 mM KCl (stripped columns) in 5% sugar solution or sugar solution alone (interval+; stippled columns) for 12 h before training. Another two groups of flies, fed sugar solution alone, was subjected to another, basically similar procedure except that there was no 5-min rest interval between initial and reversal training (interval−; crossed columns). When compared with the corresponding AIs during initial training, which did not differ from each other [$F(2,$

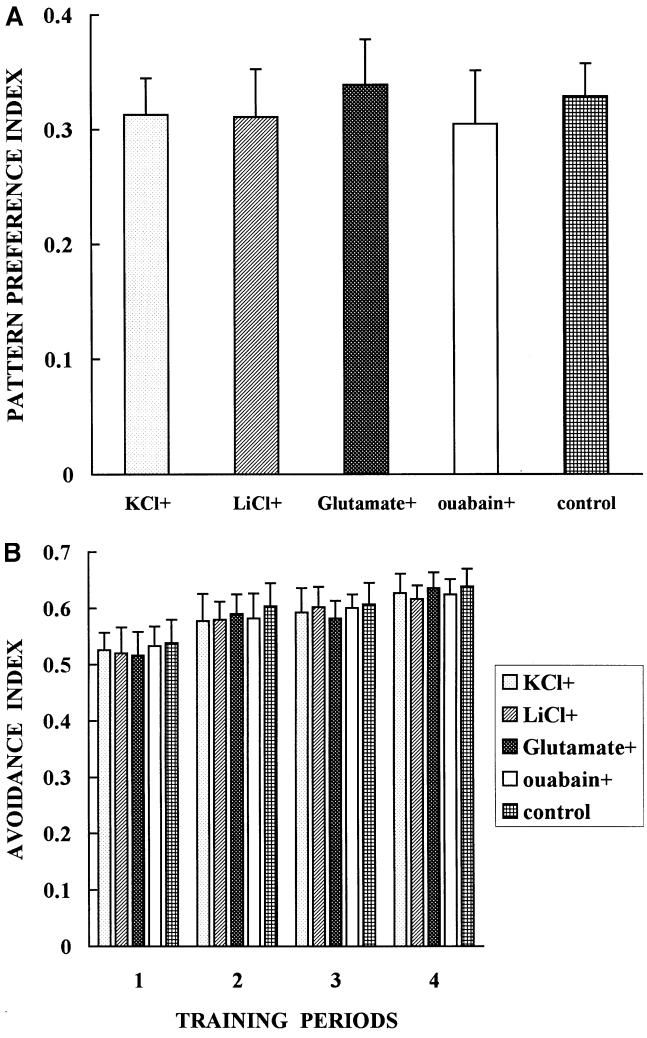

FIG. 5. Pattern preference indices and avoidance indices of flies subjected to five different feeding regimens. A: PPIs of the flies. $N = 20$ flies for each group. B: AIs of the flies during the four training periods. $N = 8$ for each group.

24) = 0.03, $p > 0.2$], reversal training produced a similar AI in the KCl-fed flies [$t(16) = 0.17$, $p > 0.8$] but significantly reduced the two AIs in the control (i.e., interval+ and interval−) flies [$t(16) \geq 3.12$, $p < 0.01$]. The KCl-fed flies acquired the heat-pattern association presented in reversal training, producing a learning index immediately after reversal training similar to that measured immediately after initial training [$t(16) = 0.29$, $p > 0.8$]. Nevertheless, memory retention was near zero when tested at 5 min after reversal training [$t(8) = 1.77$, $p > 0.1$]. In interval+ group, learning acquisition was only significantly reduced by reversal training when compared with that measured immediately after initial training [$t(16) = 3.35$, $p < 0.01$], and memory was detected [$t(8) = 4.17$, $p < 0.01$ for interval+ vs. zero]. The flies in the interval− procedure seemed to acquire the heat-pattern association presented in reversal training, producing a reduced learning index (shown as 0.14 ± 0.04) immediately after reversal training. However, they failed to form memory [$t(8) = 0.87$, $p = 0.4$ for interval− vs. zero].

DISCUSSION

In a study of inhibitors of memory that act during training and interfere with the brain in so many ways, it is necessary to separate the behavioral deficits due to disordered memory formation from those caused by nonspecific effects of inhibitors on flies' visual perception or heat sensation or of learning performance necessary for displaying normal memory retention to confirm an effect on memory. With this learning paradigm, only when the flies can normally perceive and recognize the visual patterns, sense heat reinforcement and associate heat with the punishment pattern, do they show normal spontaneous pattern preference during the pretraining session and behavioral performance during training (47).

PPI is defined as the maximal absolute PI ($|PI|_{max}$) of the three PIs during the pretraining session. Learning experiments without any pretraining lead to lower learning scores (17). Probably, a fly has to become familiar with the flight conditions in the flight simulator, i.e., to learn how to stabilize the panorama in closed loop. If the flies become more active or cannot recognize the visual patterns used as landmarks (9,42) efficiently, they change the flight directions from "nonheated" quadrants to "heated" ones more frequently or vice versa, leading to reduced PPIs (46). Therefore, if the employed drugs have exerted some nonspecific effects on the flies' visual recognition or discrimination, the animals should be unable to stabilize the panorama or choose their preferred pattern well enough to show normal PPIs (17). The disruptive drugs all produced no effect on flies' spontaneous pattern preferences, indicating that the visual perception and discrimination are undisturbed.

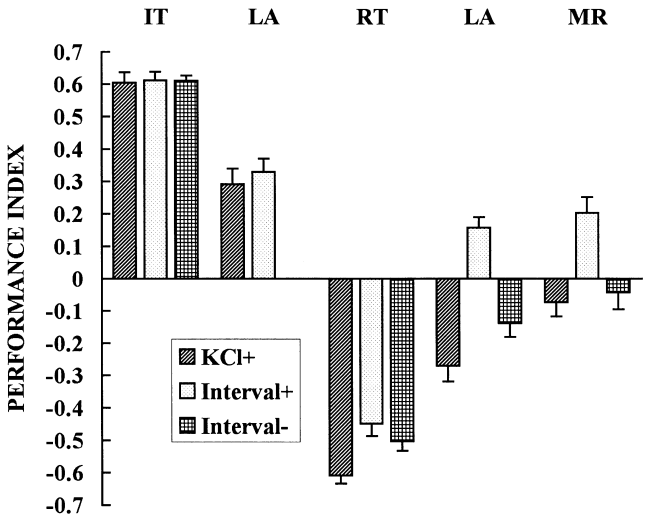

FIG. 6. Reversal training of the KCl-fed flies. Different groups of flies were subjected to reversal training with the reversal conditioning procedure described in Methods and one derivative procedure. IT, initial training; RT, reversal training; LA, learning acquisition; MR, memory retention. $N = 9$ for all groups.

It is complicated to interpret flies' behavioral performance during training because the fact that flies do not change their flight directions from the "nonheated" quadrants to the "heated" ones or vice versa also may be due to other reasons (e.g., inactivity, spontaneous pattern preference or spontaneous change of pattern preference). Nevertheless, behavioral performance of flies during training represents at least the avoidance behavior of heat and learning performance (47). The avoidance behavior is the measure of the ability of flies to avoid heat punishment. The learning performance is the measure of increment in avoidance indices (i.e., referred to the increment of AIs) with the training time. The result, that all flies fed with the used inhibitors produced the same avoidance indices as the control flies, indicates that these flies can avoid the heat-associated pattern efficiently, associate it with heat (learning), and improve their avoidance behavior based on their acquired "experience" from the preceding training as normal wild-type flies (42,47). In addition, these drugs exerted no effect on learning acquisition measured immediately after training. Therefore, these observations suggest that all drugs used here act by relatively specific biochemical mechanisms but not by rough disorganization of brain function and leave the only reasonable explanation of their effects to be a specific disruption of memory.

In the flies fed the depolarizing drugs, memory was absent very soon after training. This apparent retention deficit also could be due to impaired retrieval mechanism(s). However, this suggestion may be excluded by the following facts. During reversal training, all control flies produced significantly lower AIs than naive animals (see AIs in initial training). When a 5-min rest interval was introduced between initial and reversal training, the flies (interval+) appeared not to "give up" the acquired heat-avoidance behavior from initial training, although their learning acquisition was significantly reduced by reversal training. Without the 50 min rest interval, the flies (interval−) changed their "pattern preference" and yielded one reduced learning index immediately after reversal training. Nevertheless, they failed to form memory when tested at only 5 min after training. These results confirm our previous suggestion that memory may be formed within the first 2 min after training and, if formed, cannot be extinguished completely by the following reversal training (46). They also indicate that reversal training interferes with memory formation when introduced immediately following initial training. However, the heat-pattern association presented in initial training may exert a "diminishing" effect on flies' learning performance to reduce AIs during reversal training. The KCl-fed flies performed the same as naive wild-type flies during reversal training and acquired the heat-pattern association presented in this training session. This result suggests that the KCl-feeding regimen may disrupt memory formation but not retrieval. Otherwise, the heat-pattern association acquired

from initial training must be coded in the brain of the flies, which should interfere with the flies' conditioned performance during reversal training as occurred in control experiments.

A transient decrease in memory retention occurred within 1–3 min after training. This result is reminiscent of the Kamin deficit (22) and the similar report about memory after olfactory associative learning in flies (11). In multiphasic pathway of memory consolidation, this retention deficit has been interpreted as a transition from one memory phase to the succeeding phase (13,26). If this notion is to be accepted, a memory phase should be available about 3 min after training. This prediction was confirmed by the result that the depolarization drugs exerted no effect on learning acquisition measured in one 3-min test session immediately after training but abolished memory starting after 5 min. Because ouabain abolished memory later than 30 min after training, it should disrupt the formation of a later memory phase. Our previous work has suggested at least three memory phases after operant conditions: (a) an anesthesia-sensitive memory (ASM) phase that lasts about 20 min after training and can be disrupted by cold anesthesia, (b) an anesthesia-resistant memory (ARM) phase that is available in a time interval of 20–150 min after training and is insensitive to cold anesthesia and cycloheximide (CXM) and (c) a long-term memory (LTM) phase is activated at least 150 min after training and can be disrupted by CXM feeding (47). In addition, ASM may include two memory phases (Xia et al., unpublished data): a very short-term memory and a short-term memory (STM) that may be present later than about 2 min following training. Thus, we assume that the depolarizing drugs disrupt the formation of STM, whereas ouabain disrupts the formation of ARM. This hypothesis appears to be more reasonable in monosodium glutamate experiments in which the drug abolished memory soon after training but exerted less effect on memory after 30 min.

The multiple-phase model of memory consolidation after operant conditioning agrees broadly with the hypothesis that memory formation involves an intricate, multiple-phase consolidation pathway that has been suggested independently by many other researchers using different species and tasks (3,8,13,18,38). The similarity in consolidation of memory formation greatly extends the argument that the basic mechanisms underlying learning and memory broadly appear to be common among different tasks in flies and among different species across the animal kingdom. As argued previously, a mechanism also may exist in flies after operant conditioning (47), which deals with neural modifications in ionic conductance following conditioning and appears to operate in several invertebrate and vertebrate models [for reviews, see (2,10, 23,35,43)]. This mechanism postulates that neural modifications of preexisting synapses following conditioning seem to be due to changing transmitter release from related neuron terminals by modifying the conductance of certain ion channels and lead to enhanced activity of a specific neural pathway.

Mutational and pharmacological alterations of neuronal membrane function have been shown to disrupt the formation of STM after courtship or olfactory classical conditioning (5,6). The mutations are *shaker*, with the alternated kinetics of action potential-dependent K^+ conductance (32) and increased neuronal excitability (34), *eag*, with the reduced rectification K^+ current (45), and *Nap*^{ts}, with the decreased neuronal excitability most likely by specifically disrupting Na^+-channel function (44). Although the present experiments do not identify the biological mechanism of memory formation in any unequivocal fashion, the present results are consistent with the mechanism of associative learning about neural modifications in ionic conductance. It may be deduced with due caution that the depolarizing drugs may disrupt memory by changing relevant ion-channel function. There are at least two possible ways through which the drugs would interfere with neuronal mechanisms underlying memory formation. First, the drugs may directly interfere with some biochemical event(s) associated with memory. High K^+, for example, can reduce protein phosphorylation through mechanisms that are poorly understood (25). Therefore, KCl might interfere with changes in ionic conductance following conditioning so as to disrupt memory by affecting phosphorylation of proteins associated with related ion channels. The second possibility is that the drugs may induce some changes in neuronal function such as increasing membrane excitability or neuronal activity to induce amnesia indirectly. The depolarizing drugs, for example, may cause neuron terminals that increase their permeability to Ca^{2+}, accumulate Ca^{2+} and release transmitter under some physiological conditions (4). Considering that the drug-fed flies could learn as well as control flies, the effect of the drugs appears to be a level of physiological disruption at which normal learning can still occur.

It is also noticeable that monosodium glutamate appears to exert a less diminishing effect on memory than KCl and LiCl. As the most abundant excitatory neurotransmitter in the central nervous system, glutamate activates a variety of receptors, including G-protein-coupled metabotropic (mGlu) receptors that indirectly regulate electric signaling and activate various second-messenger cascades. This latter property makes mGlu receptors ideal candidates to translate a short neuronal activation into long-lasting intracellular changes that are widely believed to underlie processes of learning and memory [for reviews see (28)]. Recent work has suggested that activation of mGlu receptors might be required during learning and memory consolidation (27,29). In addition, retention of the fear-conditioned response has been shown to be significantly reduced in $mGlu_1$-mutant mice (1). These investigations reinforce the idea that glutamate receptors are involved in learning and memory. Therefore, monosodium glutamate also might disrupt memory by changing the relevant function of mGlu receptors. Feeding flies with monosodium glutamate before training, for example, might cause pretraining activation of mGlu receptors, which may interfere with learning and memory (27).

The principal known action of ouabain is its inhibition of Na^+/K^+ ATPase, which thereby blocks the active transport of sodium and potassium across cell membrane (12,16). Biochemical consequences of interference with membrane transport may produce various perturbations of cell metabolism, any of which might cause memory disruption. Ouabain may have disruptive effects on protein synthesis, presumably by affecting ionic conditions or by inhibiting the incorporation of amino acids with protein (12,39), which is one end result ouabain shares with CXM. However, its disruptive effect on memory is so different from that of CXM (47) that any shared biochemical actions they might have cannot explain ouabain's interference with ARM. The disruptive effect of ouabain on memory in flies is essentially the same as that in chicks or rats trained with various tasks (13,14,30); in chicks, ouabain has been assumed to induce amnesia through inhibition of sodium pump activity (13,26). Thus, the inhibition of sodium-potassium interchange maybe the important effect of ouabain on memory. Because the active transport of sodium and potassium is blocked, nerve cells firing action potentials would accumulate sodium and

lose potassium, some of which might be neurons normally involved in the process of memory formation.

Although a valid explanation of the disruption by the depolarizing drugs is missing and requires further experiments, the present results are consistent with a previous report suggesting that pharmacological alteration of neuronal membrane function by drug feeding disrupts the formation of STM (5) and support the notion that the functional integrity of ion channels is necessary for normal learning and memory formation (5,6,13). The overall conclusion is that operant conditioning may alter membrane excitability and synaptic activity and that STM can be disrupted by depolarizing drugs such as KCl and LiCl.

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