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Three *Drosophila* mutations that block associative learning also affect habituation and sensitization

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ABSTRACT **_Drosophila melanogaster_ has been conditioned with shock to avoid various odors. Mutants that failed to learn this task have been isolated. Here we report tests on these mutants for more elementary types of behavioral plasticity—habituation and sensitization of a reflex. Fruit flies have taste receptors on their feet. When a starved, water-satiated fly has sucrose applied to one foot, it usually responds by extending its proboscis. In normal flies this feeding reflex shows habituation: application of sugar to one foot depresses responsiveness through the contralateral leg for at least 10 min. The reflex also shows brief sensitization: application of concentrated sucrose solution to the proboscis increases subsequent responsiveness to tarsal stimulation for 2–5 min. In three associative learning mutants, the proboscis-extension reflex is present with a normal threshold but behavioral modulation of the response is altered. The _dunce_, _turnip_, and _rutabaga_ mutants all habituate less than normal flies. In addition, sensitization wanes unusually rapidly in _dunce_ and _rutabaga_ flies, lasting less than a minute in the case of _dunce_.**

Animals can modify their behavior, based on past experience, in several ways. Along with associative learning, which includes classical and operant conditioning, they show two elementary types of neurally mediated behavioral plasticity which may be considered forms of nonassociative learning. Habituation, broadly defined, is the decrease in a behavioral response on repeated presentations of the same stimulus. (If the decrease merely reflects a change in the sensory receptors it is called "adaptation.") Sensitization, on the other hand, is an increase in an animal's responsiveness after a strong or novel stimulus. Whether habituation, sensitization, and learning rely on similar neural mechanisms is currently not known. This issue has recently become important.

Associative learning corresponds to "learning" used in the educational sense, and psychological research has concentrated on this phenomenon. Nevertheless, progress in understanding the underlying mechanism has been slow. The physical and chemical changes that take place when an associative memory is stored in the brain are not known.

By contrast, habituation and sensitization look relatively uncomplicated and seem to be amenable to elucidation with present techniques. Recently, in fact, there has been rapid progress in this area. In particular Kandel and his colleagues (1–3) working with the mollusc *Aplysia*, have found that a gill-withdrawal reflex undergoes habituation and sensitization. They have been able to correlate this behavioral plasticity with changing transmission efficacy in a few parallel synapses in the central nervous system, and they have identified physiological and biochemical events that appear to underlie the synaptic changes. The general applicability of their model has not been established. It is unlikely, for example, that habituation proceeds by the same mechanism in all cases. Nevertheless, other workers (4–8) have found that similar processes underlie synaptic modulations in various systems which suggests some generality for the picture from *Aplysia*.

If we had evidence relating elementary plasticity to associative conditioning, then insights into habituation and sensitization could be applied to associative learning. Genetics can provide this evidence. Finding mutations that disrupt two behavioral patterns at once indicates that the mechanisms underlying the patterns are related. For example, if a single-gene mutation blocks both habituation and associative learning (but leaves other behaviors unaltered), these two types of plasticity must share components because they depend on a common gene product.

A logical organism for such a study is the fruit fly *Drosophila melanogaster*, for which associative learning mutants are at hand. Populations of wild-type *Drosophila* can learn to avoid a specific odor after experiencing it in conjunction with electric shock (9). Single-gene mutants that fail to learn in this test (10, 11) or that forget rapidly (12), although their behavior is apparently normal in other respects, have been isolated. Some of these mutants also do poorly in a different task—operant conditioning of leg flexion to avoid shock (13). We decided to test these learning mutants for habituation and sensitization of a reflex response.

Flies can taste with their feet. If a hungry, water-satiated fly has its tarsal chemoreceptors stimulated with sugar solution, it extends its proboscis, beginning a behavioral sequence that normally culminates in feeding. Proboscis extension is a reflex; it does not depend on feeding reinforcement. However, it is subject to various forms of modulation. These were recognized by Dethier (14) as instances of behavioral plasticity and have been extensively characterized in larger flies by him and others (15–17). In this paper we show that, in normal *Drosophila*, this reflex is subject to brief sensitization: application of concentrated sucrose solution to the proboscis arouses the fly for 2–5 min, causing it to respond to previously ineffective stimuli such as water. We also demonstrate long-lasting, centrally mediated habituation: brief application of sugar solution to one foot will depress responsiveness to a threshold stimulus applied 10 min later to another foot. We also examined the learning mutants for abnormalities in sensitization and habituation.

MATERIALS AND METHODS

Fly stocks and culture methods

D. melanogaster of the Canton-Special (C-S) wild-type strain and six mutant derivatives were used. The mutants *dunce*[1] (10),

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Abbreviation: HI, habituation index.

dunce^2 (18), *cabbage*^{PS264} (11), *turnip*^{PS274} (12), and *rutabaga*^{PS511} (11) were originally isolated (methods of ref. 10) because they consistently gave low learning scores in an olfactory discrimination paradigm (9). Mutant *amnesiac* flies learn normally in this test but show abbreviated memory retention (12). The *dunce*, *turnip*, *amnesiac*, and *rutabaga* mutations have been genetically mapped (10, 12, and 13; unpublished data); *cabbage* has merely been localized to the X chromosome. All these mutations (except the two *dunce* alleles) complement one another, indicating that they alter different genes (unpublished data). Flies were maintained at 25°C and 40% relative humidity on standard cornmeal medium (19) in half-pint milk bottles. Approximately equal numbers of male and female flies of each genotype were tested. Flies were tested 3–5 days after eclosion.

Statistical significance

All variation between groups was tested with one-way analysis of variance. Individual comparisons were made with Student's *t* test or, when applicable, paired Student's *t* test.

Measurement of habituation

Preparation of Flies. Between 50 and 100 flies of a given genotype were starved for 21 hr at 25°C in a clean half-pint milk bottle containing 10 ml of water on four folded Kimwipe tissues. To avoid experimenter bias, the genotypes were coded and observations were made without knowledge of the code. After starvation, about 16 flies from a bottle were anesthetized with CO_2, placed under a dissecting microscope, and individually attached to "lollipops"—small (1 cm) Tackiwax discs on wooden sticks. A fly was affixed by placing it dorsal side down on the disc, spreading its wings, and sticking them to the wax with Dumont no. 5 forceps. Small bits of wax over the wingtips and mesothoracic tarsi held the body and middle legs immobile but left the fly free to move its other legs and proboscis unhindered. Most flies recovered movement within 5 min after anesthetization; those that did not were discarded. Flies to be tested were held for 1–2 hr in a humidor (22°C, >95% humidity) to recover fully.

Testing. A fly on its lollipop was placed under a stereo-microscope (×20) with its head pointing away from the experimenter. Our procedure consisted of a series of steps in which a fly's prothoracic legs were touched with drops of distilled water, dilute (4 mM) sucrose, or concentrated (0.1 M) sucrose. Plastic syringes (10 ml) with 1.3-cm 27-gauge hypodermic needles (0.25 mm o.d.) were used to deliver all solutions. The size of the drop on the end of the needle was adjusted by moving the syringe plunger.

Flies were satiated with water before each sucrose stimulation. A relatively large (≈0.5 mm) drop of water was brought in on a needle until it simultaneously contacted both prothoracic tarsi and the proboscis. This contact was maintained for 2 sec or until the fly stopped drinking and retracted its proboscis, whichever was longer.

Water test. This was performed after satiation. The fly was checked for thirst or general excitability by applying a small drop of water to the foot about to be stimulated with sucrose. The drop size was adjusted so that the water just covered the beveled inside tip of the needle, permitting very localized contact with the leg. The needle tip was brought down until the drop touched the two most distal segments of the prothoracic tarsus. Contact was maintained for 2 sec. If the fly responded with proboscis extension, the satiation and water test were repeated. If it repeatedly extended its proboscis to water alone, it was discarded.

Initial test stimulus. A 4 mM sucrose solution (near threshold) was used. A small drop on the inside of a needle tip was brought into contact with the two most distal segments of the left prothoracic tarsus. Contact was maintained for 2 sec, the fly was observed, and proboscis extension, if any, was noted. ["Proboscis extension" corresponded to position 2 or greater on the visual scale of Dethier *et al.* (figure 1 of ref. 16)]. The leg was then rinsed with water to remove any adhering sugar. A large (≈0.5 mm) drop of water was pressed to the tibia of the leg until it surrounded the cuticle and then slid down and up the tibia and tarsus and removed, leaving the leg apparently dry. The two prothoracic legs rarely (<1% of the tests) contacted each other between sugar stimulation and water rinse.

Habituating stimulus. Concentrated sucrose was applied between the initial and final tests. The habituating sequence—consisting of satiation, water pretest, sucrose stimulation, and water rinse—was the same as in the initial test except that (*i*) 0.1 M sucrose was used, and (*ii*) the solutions were applied to the right prothoracic leg.

Final test. This was identical to the initial test, except that the water rinse afterward was omitted. After the final test, the fly was given a post-test to measure its viability and responsiveness. A large (≈0.5 mm) drop of 0.1 M sucrose was touched simultaneously to both prothoracic tarsi for 2 sec. Flies (≈5%) that failed to extend their proboscises to this strong stimulus were considered unhealthy and excluded from the data.

Habituation was usually tested by using 10-min intervals between the initial test, habituating stimulus, and final test. Flies were tested in groups of 10–16 and each stimulus was applied to all the flies in order. Each fly was used for only one habituation sequence.

Sham-habituation control experiments. The procedure was the same as for habituation, except that the actual application of the 0.1 M sucrose during the habituating stimulus sequence was omitted.

One-minute habituation experiments. These were similar to those above except that the habituating stimulus and between-test intervals were changed. The initial test stimulus of 4 mM sucrose to the right prothoracic leg was followed at 1-min intervals by three more 4 mM stimulations of the right leg and the final test. Thus, a complete procedure consisted of a water test, five identical presentations of 4 mM sucrose to the right prothoracic leg, and a post-test with 0.1 M sucrose.

Measurement of sensitization

Preparation of Flies. Between 50 and 100 flies of a given genotype were starved as in the habituation assay above. Again the genotype was coded and the behavioral assays were done "blind." Ten to 20 starved flies were anesthetized with CO_2 and attached to wax lollipops as above, except that all legs were left unrestrained. Thin double-edged razor blades were cut with scissors to give several pieces about 7 × 7 mm, each with a rounded notch about 2 mm wide and deep, on one side.

A piece of razor blade was placed with the notch over each fly's neck so that head and thorax were walled apart and the proboscis could not touch the legs. Flies (≈5%) that failed to move normally within 5 min after anesthetization were discarded. The rest were held for 1–2 hr in a humidor to recover completely.

Testing. A fly on its lollipop was placed under a stereomicroscope (×31). Two drops of water (≈0.2 mm in diameter) were placed on opposite sides of the razor blade, touching the prothoracic tarsi and extended proboscis, and the fly was allowed to drink until it retracted its proboscis. After satiation, the drops were removed from the razor blade with the corner of a Kimwipe tissue, and the flies were checked for thirst and general excitability by touching both prothoracic legs with a large (≈0.5 mm) drop of water. Flies that extended their pro-

boscises were resatiated; those that persisted (≈5%) were discarded. Satiated flies were left undisturbed for 2 min before the next step.

A sensitizing stimulus, very concentrated sucrose solution, was administered to proboscis chemoreceptors without allowing ingestion as follows. A syringe, containing 1.0 M sucrose, with a Tuohy adapter (Clay–Adams, Parsippany, NJ) was fitted with a glass micropipette (≈2 cm long; shank diameter, 1 mm; tip diameter, 75 μm). Pressure in the syringe was adjusted so that the surface of the sucrose solution was slightly convex across the pipette tip but did not form a drop. Under the microscope the micropipette tip was brought down until the sucrose solution touched a few lateral hairs on the labellum of the proboscis, eliciting proboscis extension and exposure of the maxillary palps (small flaps with chemoreceptive hairs on the sides of the basal proboscis). The pipette was then positioned over one palp until the sucrose solution touched about one-fourth of its surface. This contact was maintained for a total of 2 sec.

Sham-sensitized control flies were treated exactly as above, but with water replacing the 1 M sucrose.

The fly was tested for sensitization at various times between 15 sec and 2 min after the sensitizing stimulus by placing a drop of water (≈0.2 mm in diameter) on the razor blade within reach of the fly's feet. Immediately, both prothoracic tarsi (and often the mesothoracic tarsi) were contacted by the drop. Visually observed proboscis extension within 2 sec after contact with the water was scored as a positive response.

RESULTS

Habituation

Ordinarily we tested our flies with a threshold sucrose stimulus, before and after application of a more concentrated sucrose stimulus to the contralateral leg. If the intervening stimulus induces habituation, it should produce a subsequent response decrement. Accordingly, for purposes of comparison, we define a numerical habituation index (HI) as the fraction of flies responding by proboscis extension to an initial stimulus with 4 mM (threshold) sucrose minus the fraction responding to this stimulus 10 min after the intervening habituating stimulus of 0.1 M sucrose. HIs for various fly stocks, given in Table 1, are reported as the mean of the scores for all groups tested ± SEM. Four conclusions follow from the data.

There Is a Response Decrement in Normal C-S Flies. In the initial test with 4 mM sucrose, 70% of the flies extended their proboscises. Ten minutes after a habituating stimulus of concentrated sucrose had been applied to the contralateral leg, only 46% of the flies responded (HI = 0.23 ± 0.02). We know that the decreased responsiveness in the second test was not due to lessened hunger because the flies were never allowed to drink sugar solution during the experiment. It was not due to sugar droplets left on the sensory hairs because the leg was rinsed with water after each contact with sucrose solution. It was also not brought on by fatigue or debility. Sham-habituated control groups (tested as above but with water instead of 0.1 M sucrose applied to the right prothoracic tarsus between initial and final tests) showed an apparent increase in responsiveness (HI = −0.02 ± 0.03), probably because of increasing hunger as the experiment proceeded. The fact that 95% of the flies responded to 0.1 M sucrose at the conclusion of the experiment also makes fatigue unlikely.

Table 1. HIs of normal and mutant *Drosophila*

Genotype	Flies, no.*	Initial responsiveness	HI (10 min)	HI (sham)
Normal	676 (48)	0.71 ± 0.03	0.23 ± 0.02	−0.02 ± 0.03
dunce	409 (28)	0.76 ± 0.05	0.13 ± 0.03†	−0.05 ± 0.03
turnip	405 (28)	0.71 ± 0.03	0.13 ± 0.03‡	−0.05 ± 0.02
rutabaga	371 (28)	0.67 ± 0.05	0.10 ± 0.05‡	−0.02 ± 0.02
amnesiac	302 (25)	0.46 ± 0.04	0.14 ± 0.03†	−0.07 ± 0.02

HI values are mean ± SEM for all groups tested.
* Number of groups shown in parentheses.
† For difference from normal, $P < 0.02$.
‡ For difference from normal, $P < 0.01$.

The Response Decrease Is Mediated by the Central Nervous System. In our experiments, application of a sucrose stimulus to the right prothoracic tarsus caused decreased responsiveness to a subsequent stimulus to the left prothoracic tarsus. This interaction between stimuli to contralateral legs must take place centrally. Adaptation of sensory receptors on the left leg cannot be responsible because sham-habituated control groups (also tested twice on the left leg) showed no response decrement.

The Decrease in Response Is Specific to the Sensory Channel Used to Produce the Habituation. In other words, after stimulation with sucrose, the flies become less responsive only to further sugar stimulation, not to all stimuli. Our procedure allowed us to check for this channel specificity because we routinely stimulated another sensory channel of the flies—the tarsal water chemoreceptors—in our pretests before each application of sugar. Immediately before the habituating stimulus, 50 ± 5% of the flies extended their proboscises in response to water. Ten minutes later, just before the final test, 47 ± 4% responded. This difference is not significant.

Three Single-Gene Mutants, Originally Selected for Failure in Associative Learning, Are Relatively Deficient in Habituation. *Dunce*, *turnip*, and *rutabaga* flies showed abnormally low HIs at 10 min (Table 1).

Several artifacts that might explain the mutants' abnormal scores have been ruled out. In all tests, before any sucrose stimulation, the flies were given water until they failed to extend their proboscises. This should prevent false responses due to thirst or sensitization. Moreover, the three mutants behaved indistinguishably from normal flies in the sham-habituation controls, had the same probability of response in water pretests, and showed normal viability, responsiveness, and sensory thresholds.

A fourth mutant tested, *amnesiac*, also showed a lower than normal HI, 0.14 ± 0.03. The interpretation of this result is ambiguous, however, because this mutant showed somewhat less than normal native responsiveness. If response decrease is measured as a fraction of initial responsiveness, then *amnesiac*'s habituation is similar to that of normal flies. Therefore, no firm conclusions are possible in the case of this mutant. A fifth learning deficient mutant, *cabbage*^{PS264} (11), showed very low and unreliable native responsiveness to sucrose and so was not tested further.

Note that decreased habituation corresponded to increased responsiveness in the final test. This effect could not logically result from lethargy or debility. In summary, the abnormal habituation indices observed in *dunce*, *turnip*, and *rutabaga* were not due to obvious differences in excitability, sensory acuity, or vitality; they appear to reflect genuine abnormalities in behavioral plasticity.

In our usual habituation procedure, a single concentrated (0.1 M) sucrose stimulus applied to the contralateral leg produced a strong, relatively long-lasting (10 min) response decrement. This allowed rapid testing of many flies, with an internal control for sensory adaptation. Nevertheless, it differed from the traditional habituation procedure in which repeated presentations of identical stimuli bring about the behavioral

Table 2. Responsiveness of sensitized normal and mutant *Drosophila*

Genotype	Flies, no.*	Fraction of flies responding at various times after sensitizing stimulus							
		At 15 sec		At 30 sec		At 60 sec		At 120 sec	
		S	S − C	S	S − C	S	S − C	S	S − C
Normal	699 (52)	0.49 ± 0.05	0.34 ± 0.05	0.43 ± 0.08	0.27 ± 0.08	0.50 ± 0.04	0.35 ± 0.04	0.36 ± 0.07	0.23 ± 0.06
dunce	523 (41)	0.59 ± 0.05	0.50 ± 0.04†	0.45 ± 0.06	0.37 ± 0.07	0.18 ± 0.05†	0.13 ± 0.06†	0.18 ± 0.07‡	0.08 ± 0.05
turnip	682 (43)	0.45 ± 0.07	0.38 ± 0.08	0.36 ± 0.07	0.28 ± 0.06	0.39 ± 0.07	0.29 ± 0.08	0.28 ± 0.07	0.22 ± 0.07
rutabaga	626 (47)	0.44 ± 0.07	0.38 ± 0.07	0.22 ± 0.06	0.19 ± 0.06	0.25 ± 0.04†	0.21 ± 0.05§	0.12 ± 0.05‡	0.08 ± 0.04§
amnesiac	574 (43)	0.52 ± 0.08	0.41 ± 0.10	0.27 ± 0.06	0.25 ± 0.05	0.24 ± 0.05†	0.18 ± 0.06§	0.23 ± 0.06	0.16 ± 0.06

Fractions are mean ± SEM for all groups tested. S, responsiveness of sensitizied flies; S − C, change in responsiveness attributable to sensitization (i.e., the fraction responding for sensitized flies minus the fraction responding for the appropriate sham-sensitized controls). S − C values were calculated for each group before averaging.

* Number of groups shown in parentheses.

† For difference from normal, $P < 0.01$.

‡ For difference from normal, $P < 0.02$.

§ For difference from normal, $P < 0.05$.

change. Therefore, we tested normal C-S flies and two of our mutants with five consecutive 4 mM sucrose stimuli applied to the same leg at 60-sec intervals (1-min habituation). The C-S flies showed response decrement (HI = 0.18 ± 0.02); *dunce* and *turnip* showed significantly less (*dunce*, 0.10 ± 0.03, $P < 0.05$; *turnip*, 0.05 ± 0.02, $P < 0.01$). Thus, these mutants showed abnormally low levels of response decrement in two habituation procedures incorporating different controls and measuring different retention times.

Sensitization

In these experiments our water-satiated flies ordinarily responded to water only after a sensitizing stimulus of 1 M sucrose applied to the proboscis. Nevertheless, there was always a small background responsiveness due to thirst or nonspecific arousal. To control for this, half the flies in any group were "sham-sensitized"—they had water instead of 1 M sucrose applied to their proboscises. Sham-sensitized flies of all genotypes showed similar low levels of responsiveness, with about 10% of tested individuals responding.

After a sucrose sensitizing stimulus, normal flies showed high (≈50%) responsiveness for about 60 sec after exposure to 1 M sucrose, with a gradual decline thereafter. Table 2 shows uncorrected responsiveness for sensitized flies of various genotypes, and also the increase in responsiveness which is specifically attributable to sensitization; i.e., responsiveness of sensitized flies minus responsiveness of sham-sensitized controls. The waning of sensitization with time is plotted for normal and mutant flies in Fig. 1. The *turnip* mutants behaved indistinguishably from normal flies. Mutant *dunce*[1] flies, on the other hand, showed a transient sensitized state, with greater-than-normal sensitization at 15 sec followed by a rapid decline until, at 60 and 120 sec, sensitization was significantly lower than normal. Sensitization was also somewhat abbreviated in *rutabaga* and *amnesiac* flies. These mutants showed a normal level of sensitization at 15 sec, but the effect decayed rapidly thereafter; it was significantly below normal at 60 sec.

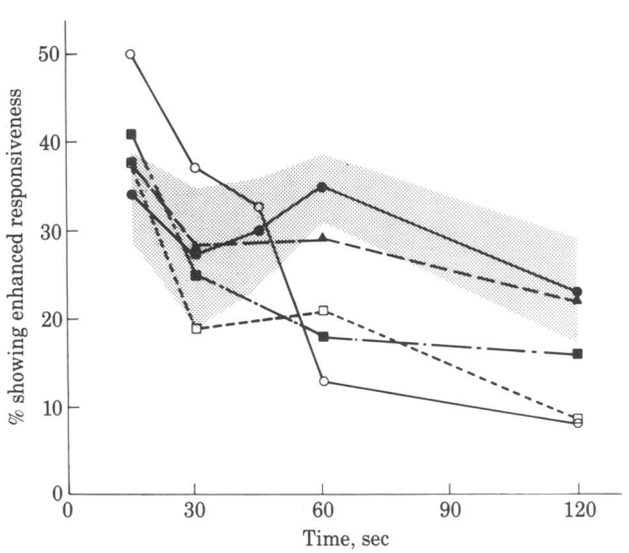

FIG. 1. Decay of sensitization in normal and mutant flies, shown as percentage of sensitized flies responding in water test minus percentage of sham-sensitized controls responding plotted against time between sensitizing stimulus and water test. ●, Normal flies; ○, *dunce*; ▲, *turnip*; □, *rutabaga*; ■, *amnesiac*. Shading indicates SEM for normal flies.

We were careful to ensure that the enhanced responsiveness in sugar-stimulated flies was due to sensitization, not associative learning. Because a water stimulus to test for thirst was given before sucrose was administered to the flies' proboscises, we worried that they might become classically conditioned to respond to water (20).

To prevent such an effect, we separated the initial water-satiation test from the sucrose stimulus by 2 min, much longer than the interval needed to preclude association in both *Phormia* classical conditioning (20) and *Drosophila* olfactory learning. Care was also taken to prevent conditioning to visual cues. During water tests to the legs, the needle applying water to the tarsus was kept nearly horizontal, hidden from the fly's view by the razor blade over its neck. Our flies had no opportunity to associate tarsal water stimulation with oral sucrose. Thus, the altered responsiveness of *dunce*, *rutabaga*, and *amnesiac* in these experiments must result from abnormal sensitization.

DISCUSSION

The sensitization we measured after sucrose stimulation is apparently identical to the "central excitatory state" previously observed by Dethier *et al.* (16) in the blowfly *Phormia regina*. Our experimental procedure parallels theirs, and the time course of the effect is similar in the two species.

Dethier (15) also demonstrated a centrally mediated response decrement in *Phormia*, analogous to that reported here for *Drosophila* but much briefer, lasting less than 15 sec. It may be that the kinetics of the response decrement are different in the two species. It is also possible, however, that the central excitatory state masks habituation between 15 sec and about 2 min after training, by producing the opposite tendency—enhanced responsiveness—at these times.

Four of our mutations affect nonassociative as well as associative learning. Mutant *turnip* flies show abnormally low habituation of the proboscis extension reflex, although sensitization of this response is normal. The *dunce* and *rutabaga* mutants show both low habituation and brief sensitization. Mutant *amnesiac* flies appear to have low habituation, and they show abbreviated sensitization, although this interpretation is weakened because of *amnesiac*'s slightly increased threshold for sucrose. To summarize, one of our mutations alters two types of behavioral plasticity, and two others affect all three. A fourth mutant, *amnesiac*, is probably affected in sensitization. These results suggest that the mechanisms underlying these behaviors have similarities, because they rely on some of the same gene products. On the other hand, the fact that *turnip* flies show normal sensitization suggests that the processes have dissimilarities. Moreover, all the mutants we tested affect associative more than nonassociative learning. In comparison with the dramatic curtailment in olfactory discrimination learning by which they were isolated, the mutations' effects on habituation and sensitization are relatively weak. Also, it must be pointed out that in most cases we do not know what the affected gene products do in the fly, so we cannot say at present that the mutations affect mechanisms central to behavioral plasticity. They could alter accessory processes such as arousal, although the normal sensory threshold and responsiveness of *dunce*, *turnip*, and *rutabaga* are reassuring in this regard.

Our conclusions depend on the assumption that the multiple effects on behavioral plasticity in the mutant strains result in each case from a single genetic lesion. For two of our mutants there is good evidence that the assumption is true. An independently isolated *dunce* allele, $dunce^2$ (18), affects habituation, sensitization, and associative learning in the same way as the first mutation, $dunce^1$. A related argument holds for the *turnip* mutation; its effects on associative learning and habituation both map to the same genetic region (near *carnation*) on the X chromosome. Similarly, *amnesiac*'s abnormalities in sensitization and associative memory map to a locus near *carnation* (12). For *rutabaga*, we know at present only that the behavioral abnormalities are all X-linked.

The most interesting mutation is *dunce*. It affects all three forms of behavioral plasticity but leaves sensory acuity, activity levels, and motor coordination apparently intact (10). Habituation in *dunce* is depressed, although still present at 10 min. Both associative learning and sensitization are affected similarly by the mutation—they are both made transient. Dudai (21) reported evidence that *dunce* mutants can learn to associate odorant and electric shock but that the association is labile, decaying rapidly even in the first minute after training. Table 2 shows that *dunce* has a similarly brief sensitized state.

At the outset of this work we wanted to use mutations to relate elementary plasticity and associative learning by showing that they depended on common gene products. We found such evidence with the four mutants we tested. In the case of *dunce*, the affected gene product is apparently known, and the results are tantalizing. Byers *et al.* (18) have shown that one form of the enzyme cyclic AMP phosphodiesterase is decreased or absent in the mutant. The role of this enzyme in associative learning is not yet clear, but an effect on plasticity might have been predicted. Changes in neuronal cyclic AMP levels appear to underlie sensitization of the gill-withdrawal reflex in *Aplysia* (1–3).

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