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Mutations in the dopa decarboxylase gene affect learning in *Drosophila*

(serotonin/dopamine)

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ABSTRACT **Fruit flies synthesize several monoamine neurotransmitters. Dopa decarboxylase (*Ddc*) mutations affect synthesis of two of these, dopamine and serotonin. Both transmitters are implicated in vertebrate and invertebrate learning. Therefore, we bred flies of various *Ddc* genotypes and tested their learning ability in positively and negatively reinforced learning tasks. Mutations in the *Ddc* gene diminished learning acquisition approximately in proportion to their effect on enzymatic activity. Courtship and mating sequences of the mutants appeared normal, except for one aspect of male courtship that had previously been shown to be experience dependent. In contrast, the effect on behavior patterns that do not involve learning—phototaxis, geotaxis, olfactory acuity, responsiveness to sucrose—was relatively slight under these conditions. Moderate *Ddc* mutations affected the acquisition of learned responses while leaving memory retention unaltered. This is in contrast to the mutations dunce, rutabaga, and amnesiac, which primarily affect short-term memory.**

Biogenic monoamines (principally dopamine, serotonin, epinephrine, and norepinephrine in vertebrates; dopamine, serotonin, and octopamine in invertebrates) are a group of neurotransmitters formed from aromatic amino acids that seem to be related in their physiological and behavioral functions. Monoamines are implicated in synaptic modulation in a variety of animals (1–4). Numerous pharmacological studies in vertebrates indirectly but consistently suggest a role for monoamines in attentiveness and behavioral plasticity (5), particularly a role for dopamine in positively reinforced learning (6). In line with this, some neurophysiological and behavioral experiments suggest an involvement of norepinephrine in experience-dependent changes during a critical period of visual cortical development (7, 8; B. Gordon, J. Moran, P. Trombley, and J. Soyke, personal communication).

In invertebrates, there is specific neurophysiological evidence that serotonin is involved in sensitization and classical conditioning of the siphon-withdrawal reflex in *Aplysia* (9–11). Serotonin, applied to the relevant synapses in the circuit, can mimic behavioral sensitization, producing facilitation of synaptic transmission in the reflex pathway. The behavioral effect of sensitizing stimuli can be blocked by cinanserine, a moderately specific antagonist of serotonin (9). Recently, however, this story has been complicated by a failure to find serotonin-like immunoreactivity in identified facilitatory neurons [those of the L29 group (12)], although serotonergic terminals from other cells are found in the relevant synaptic sites (12).

In both vertebrates and invertebrates, therefore, the evidence linking biogenic monoamines to learning is cumulatively suggestive, but it is in no case unassailable. We decided to investigate this relation further, using *Drosophila* mutants.

Wright and his colleagues (13, 14, 15) have isolated two temperature-sensitive mutations in the structural gene for the enzyme dopa decarboxylase, Ddc^{ts1} and Ddc^{ts2}, as well as a small deficiency that deletes the gene from the chromosome. The temperature-sensitive mutations act slowly, so that a complete curtailment of enzymatic activity requires about a day at the restrictive temperature (29°C) (unpublished observation). Nevertheless, the mutations enabled these investigators to examine the function of the enzyme during development. They found that dopa decarboxylase activity was necessary at several defined times in development for cuticle hardening (16). Dewhurst *et al.* (17) and Wright (13) obtained evidence that the enzyme is present in *Drosophila* brain tissue.

This result was confirmed and extended by Livingstone and Tempel (18), who found that the *Ddc* gene product was required for the synthesis of the neurotransmitters dopamine and serotonin. They were particularly aided in their work by their discovery that temperature-sensitive mutant *Ddc* flies develop and behave normally if raised to adulthood at permissive temperature (18°C–21°C). If the temperature is then raised to 29°C, the dopa decarboxylase enzymatic activity drops to undetectably low levels in the central nervous system, allowing a clear demonstration of the necessary role of the enzyme in dopamine and serotonin biosynthesis. Surprisingly, in these circumstances the mutant *Ddc* flies not only survive but show approximately normal behavior. We decided to measure their ability in three different learning tests.

MATERIALS AND METHODS

Culture Conditions, Fly Stocks, and Crosses. All *Drosophila* stocks were bred and maintained on corn-meal agar in controlled environment rooms in a 12 hr:12 hr light/dark cycle, 40% relative humidity at the temperature indicated (20°C, 25°C, or 29°C). Ordinarily our Ddc^{+} (normal) flies were from the Canton-S wild-type stock. We also tested two other stocks of Ddc^{+} flies (provided by T. R. F. Wright), which were approximately coisogenic with the Ddc^{ts1} and Ddc^{ts2} mutants. These flies performed indistinguishably from Canton-S flies in the learning tests.

We used two different temperature-sensitive *Ddc* mutations, Ddc^{ts1} and Ddc^{ts2}, and a small deficiency for the *Ddc* region—*Df(2L)130*, hereafter denoted simply *Df*. Flies homozygous for the deficiency fail to survive, and the Ddc^{ts1} stock contained an unrelated female sterile mutation (T. Wright, personal communication). Therefore, these mutant

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Abbreviations: A, learning index; *Ddc*, dopa decarboxylase gene.

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stocks were provided and routinely maintained as heterozygotes, over the second-chromosome balancer *In(2LR)O,Cy*, hereafter denoted *CyO* (19). Stocks provided were (*i*) Ddc^{ts1} *pr sp/CyO*; (*ii*) Ddc^{ts2}/Ddc^{ts2}; and (*iii*) *Df(2L)130 pr*. The eye-color marker purple (*pr*) did not significantly affect learning in our conditions. Therefore, for simplicity, flies will be defined below simply by *Ddc* genotype. All stocks with temperature-sensitive *Ddc* mutations were kept at permissive temperature (20°C). To obtain flies with a range of dopa decarboxylase enzyme activities, we crossed Ddc^{ts1}/*CyO* flies with wild-type (+/+) and with *Df/CyO* flies. The mutation Ddc^{ts2} was viable and fertile at 20°C, so homozygous Ddc^{ts2}/Ddc^{ts2} (without markers) were maintained at 20°C and used directly. Crosses involving temperature-sensitive mutants and subsequent growth of the progeny were all done at 20°C. Flies of the desired genotype were selected from among adult F_1 progeny 1–2 days after eclosion, and they were kept at 20°C until 3 days after eclosion. At this point, some of the temperature-sensitive flies were shifted to the restrictive temperature (29°C) for an additional 3 days, while the rest stayed at 20°C. Flies of both groups were then shifted to 25°C for 18 hr, after which time behavioral assays were run at 25°C. Flies of all genotypes had their behavioral responses and their dopa decarboxylase enzyme levels measured both with and without a 3-day shift to 29°C, with the final 18 hr always at 25°C to ensure normal phototactic behavior.

Biochemistry. The L-dopa decarboxylase enzyme activity of brain homogenates was measured essentially as described previously (18). Flies were decapitated under CO_2 anesthesia, and the heads were collected in oxygenated *Drosophila* Ringer's solution (20). The head cuticle and corneas were removed from the brain and optic ganglia with a pair of Dumont 55 forceps. Decapitation and dissection of two fly brains took <10 min.

For a given assay, two brains were homogenized in 20 μl of 0.2 M sodium phosphate buffer, pH 7.0/dopamine (0.5 mg/ml)/pyridoxal phosphate (0.1 mg/ml). Five microliters of this homogenate was incubated for 30 min at 25°C with 5 μCi of L-[^{3}H]dopa (20 Ci/mmol; 1 Ci = 37 GBq) in a total reaction volume of 10 μl. The reaction was stopped by adding 4 μl of 30% formic acid containing unlabeled dopamine at 1 mg/ml. Tritiated reagents and metabolites were separated by high-voltage paper electrophoresis (18, 21), and then the paper was cut into 1-cm segments and assayed in a liquid scintillation counter. The chemical identity of the dopamine peak was confirmed as described (18) by paper chromatography in a second dimension, using the solvent system methylethyl ketone/propionic acid/water (25:8:7). Rates of reaction of this and other fly enzymes (22) were affected by the temperature at which the flies were raised. Therefore, mutant stocks were always raised and assayed in parallel with groups of wild-type flies; and mutant enzyme levels are normalized relative to co-shifted and identically treated wild-type flies, given as mean percentage ± SEM for 4–12 experiments.

Measurement of Associative Learning. Flies with various *Ddc* genotypes were tested in two-odor-discrimination tasks, one using negative reinforcement (electric shock), and the other using positive reinforcement (sucrose feeding). Conditions and experimental procedures were exactly as described (23), with additional details (ref. 24; figure 6 in ref. 25). Training and testing procedures for the two learning assays were similar; we will outline negatively reinforced training first.

Thirty to fifty flies were trained by exposing them alternately to one odorant (3-octanol) paired with a 90 V electric shock and to a control odorant (4-methylcyclohexanol) presented without reinforcement. After two such training cycles, the flies were tested without reinforcement by running them sequentially toward test tubes containing the two odorants. In this situation, normal flies avoided the shock-associated odorant 3-octanol more than the control odorant 4-methylcyclohexanol.

To control for odor bias and other nonassociative effects, we repeated the training and testing procedure with new flies, except that this time the electric shock was paired with 4-methylcyclohexanol. As before, the numerical index of learning we use, Λ, is the fraction of the population avoiding the shock-associated odor minus the fraction avoiding the control odor, averaged over both halves of an experiment.

Our procedure for training flies with positive reinforcement was similar to the training procedure described above, except that one odor of the pair was presented to hungry flies in association with sucrose, delivered by spreading a 1.0 M sucrose solution in a 1-cm wide band on the surface with the odorant. The flies were then tested without reinforcement by transporting them to the choice point of a T-maze, between arms containing 3-octanol and 4-methylcyclohexanol. In this case, the learning index used, Λ, is the fraction of the population migrating toward the sucrose-associated odorant minus the fraction migrating toward the control odorant, averaged as before for reciprocal halves of the experiment. Normal flies learned this task about as well as the shock avoidance task, but memory persisted much longer (23).

Learning indices reported here are mean ± SEM for 6–12 experiments. In all instances here, *Ddc* mutants were trained and tested in parallel with normal flies that had identical temperature-shift histories. The experimenter was ignorant of the genotype being tested.

Other Behavioral Plasticity. Experience-dependent depression of male courtship (26, 27) was measured exactly as described (26) except that we used two females per male rather than one, and the females were mobile rather than etherized. In the conditioning exposure, the 3-day-old male fly to be tested was transferred to a clean cylindrical Plexiglas courtship chamber (6 mm high × 10 mm in diameter) containing two previously mated $\hat{X}\hat{X}$ *y f* females, who tended to reject the male's attempts at courtship. The time the male spent courting (i.e., following a female or vibrating his extended wing) was recorded by an uninformed observer. For testing, the male was shifted, either immediately or 3 hr after courtship, into a new clean courtship chamber that contained two mobile *y f* $\hat{X}\hat{X}$ virgin females, and the fraction of the 10-min period spent courting was again recorded by an observer who was unaware of the male fly's genotype. As a control, male flies of various *Ddc* genotypes who were naive (i.e., who had not been previously exposed to unreceptive mated females) were tested with virgin females as described above.

Other Behaviors. The flies' fast phototactic responses were measured using the countercurrent method of Benzer (28). A group of 40–50 flies was placed in the first tube of a behavioral countercurrent apparatus (figure 1 in ref. 24) in a dark room and run repeatedly (30 sec per run) toward a GE F15T8 CW fluorescent light. Five runs with sequential shifts of the apparatus separate the flies into six fractions, from tube 0 (least phototactic) to tube 5 (most phototactic). Flies in each fraction were anesthetized and counted.

Fast geotaxis was measured by the countercurrent procedure as described above, except that the countercurrent machine was kept vertical in a dark room and the flies were fractionated on the basis of their tendency to climb upward.

Flies' responsiveness to sucrose was measured using the proboscis extension reflex (29). Individual flies were stuck by the wings to tackiwax balls on sticks, with their legs and proboscises free to move. After a 60-min acclimation period, flies were given water to satiation. They were then tested by

touching the tarsus of one prothoracic segment with a drop of sucrose solution (0.001–0.1 M) and were then watched for proboscis extension. The observer was ignorant of the flies' genotype. Threshold for a given genotype was defined as the sucrose concentration that elicited extension in 50% of the tested population. To ensure that the tested flies had generally normal behavior they were given post-tests with pure water and strong (1.0 M) sucrose. The behavior of a fly was scored only if it responded appropriately in the post-tests, extending its proboscis to strong sucrose but not to water.

We used a T-maze choice chamber to test olfactory acuity (25). Naive flies of various genotypes were placed at the choice point of a T-maze with one tube containing either 3-octanol or 4-methylcyclohexanol; the other tube contained no odorant. The flies were given 30 sec to partition themselves between the arms, and then the flies in each arm were trapped, anesthetized, and counted.

Statistics. Differences between paired samples were tested for significance with Student's *t* test (two-tailed). The Spearman rank correlation coefficient, r_s, is used where indicated.

RESULTS

Genetic lesions at the *Ddc* locus affected brain dopa decarboxylase activity in the predicted manner (Fig. 1*a*). Both Ddc^{ts1} and Ddc^{ts2} flies showed strong temperature effects on enzyme activity. Mutant Ddc^{ts1}/Df flies and Ddc^{ts2}/Ddc^{ts2} flies showed decreased enzyme levels if raised at 20°C and nearly undetectable enzyme levels if shifted to 29°C. Flies of various genotypes are arranged (Fig. 1*a*) in decreasing order of brain dopa decarboxylase activity. When we tested these genotypes for associative learning (Fig. 1*b*), we found a strong correlation between brain dopa decarboxylase enzyme activity and both the negatively reinforced ($r_s = 0.99$) and the positively reinforced ($r_s = 0.99$) odor-discrimination tasks ($P > 0.01$ in both cases).

With two *Ddc* genotypes, Ddc^{ts1}/Df and Ddc^{ts2}/Ddc^{ts2}, learning was temperature sensitive in the same way as dopa decarboxylase enzyme activity. Both positively and negatively reinforced learning were less after 3 days at the high temperature than at permissive temperature for Ddc^{ts1}/Df ($P < 0.01$; $P < 0.01$) and for Ddc^{ts2}/Ddc^{ts2} ($P < 0.05$; $P < 0.05$).

To study the effects of these mutations on memory retention, we chose stocks with intermediate amounts of dopa decarboxylase enzyme activity, so that some initial learning would be detectable. Immediately after training, both $Df/+$ and $Ddc^{ts1}/+$ (25°C) flies showed less learning than wild-type (+/+) flies. Nevertheless, once learned information was acquired by the mutant flies, it appeared to be retained for the normal time span. Memory decay rates of both mutants were indistinguishable from similarly trained Canton-S flies (Fig. 2).

[In experiments involving positively reinforced training with sucrose, we found that the *Ddc* mutants required a longer period of prior starvation (22–24 hr) than wild-type flies to show reliable learning and long-term memory. It is as if they had to be made hungrier before they would remember a food reward.]

Dopamine and serotonin, two compounds affected by *Ddc* mutations, are important neurotransmitters in vertebrate and invertebrate brains. Mutant Ddc^{ts1}/Df flies kept at 29°C appear behaviorally normal on superficial examination. Nevertheless, we wondered whether the behavioral effects of *Ddc* mutations were at all specific to learning. The most complex fly behavior patterns that do not require learning occur in courtship, the stereotyped sequence of actions by which male and female flies recognize each other as conspecifics and copulate. If a male fly is placed in a chamber with a receptive virgin female, he normally courts her vigorously,

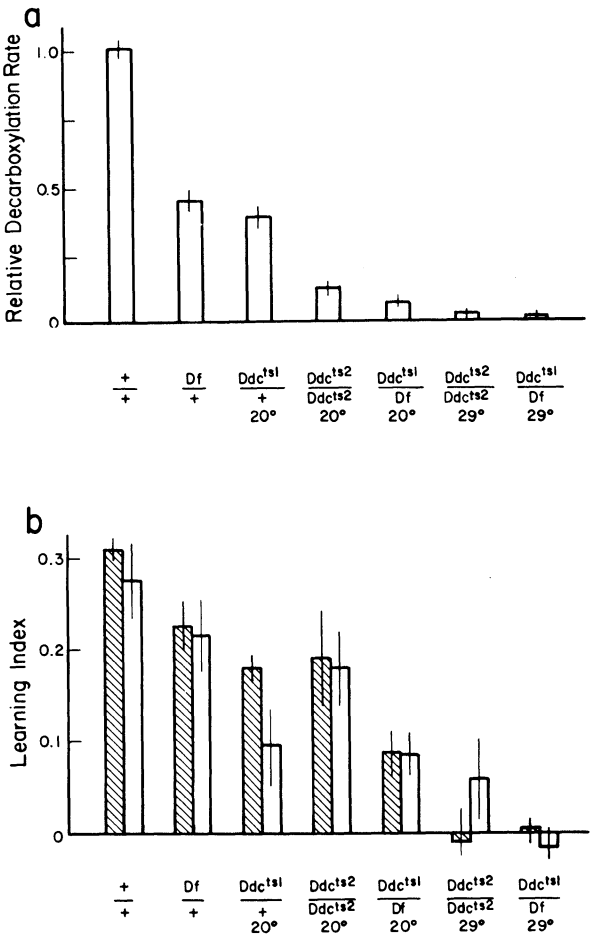

FIG. 1. Decarboxylation rate and associative learning ability in wild-type (+/+) flies and in several stocks with different combinations of *Ddc* alleles. (*a*) Dopa decarboxylase activity of *Drosophila* brain tissue was determined as described in text. Activities of the mutant *Ddc* homogenates (±SEM) are presented as percentage of activity of wild-type flies raised under the same temperature conditions. Wild-type rates were 17 ± 2 fmol per min per brain for flies raised at 20°C and 35 ± 4 fmol per min per brain for flies shifted to 29°C. Mutant *Ddc* brains had protein contents (35 mg/ml) indistinguishable from wild type (30). (*b*) Learning performance of the flies with genotypes described above, measured after training with negative reinforcement (shaded bars) and positive reinforcement (open bars).

following her around the chamber, extending and vibrating his wing in a temporally patterned species-specific courtship song. If the female is a virgin his suit is usually successful; the female eventually stops running away, and mating ensues. We tested our most severely affected mutant, $Ddc^{ts1}/Df130$ (29°C), and found that it performed all the steps of courtship and mating indistinguishably from normal flies.

Certain aspects of *Drosophila* courtship are influenced by previous experience. One such component has to do with male responses to previously mated females. These females tend to reject courtship, using a variety of behavioral and olfactory cues (26, 27). If a male fly is placed with such a female, the amount of time he spends chasing and singing to her decreases. Moreover, his subsequent ardor for courtship, even of other receptive females, remains depressed for about 3 hr (26). This courtship depression is absent in several learning mutants (26, 31), and it is abbreviated in a memory mutant (26); it has been plausibly suggested to be a consequence of learning (26, 27). Here we report that such courtship depression is absent in mutant Ddc^{ts1}/Df (29°C) flies

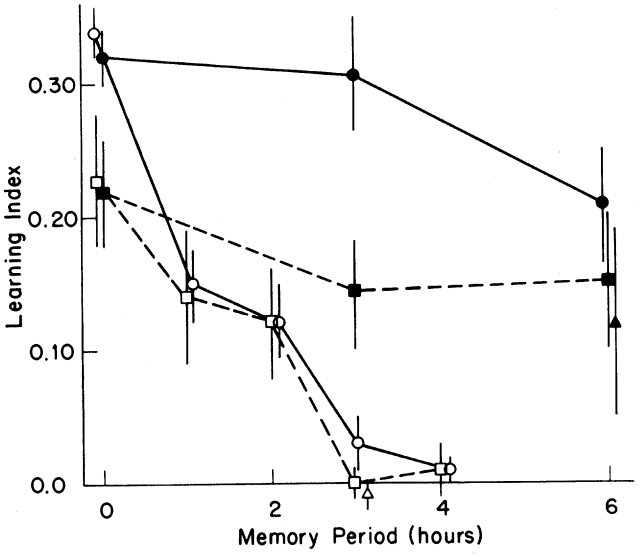

FIG. 2. Memory decay curves for wild-type flies and for moderately affected *Ddc* mutants after positive or negative reinforcement in training. Each point represents the mean learning index (±SEM) from 8–12 experiments. *Ddc* mutants tested for memory were starved 22–24 hr before sucrose training. All other reward memory was measured after 18–20 hr of starvation. ●, Wild-type flies after training with positive reinforcement; ■, *Df*/+ flies after positive reinforcement; ○, wild-type flies after negative reinforcement; □, *Df*/+ flies after negative reinforcement. To be sure that the mutant *Ddc* effect was not allele specific or due to the genetic background in the *Df* stock, we tested a second genotype, affected in dopa decarboxylase, at critical retention times: ▲, Ddc^{ts1}/+ (25°C) flies after positive reinforcement; △, Ddc^{ts1}/+ (25°C) flies after negative reinforcement.

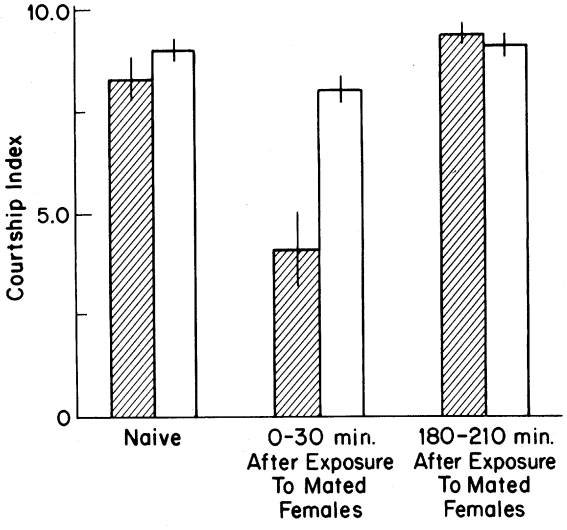

FIG. 3. Courtship behavior of wild-type flies (shaded bars) and Ddc^{ts1}/*Df* (29°C) flies (open bars). All females used were $\hat{X}\hat{X}$ *y f*; their yellow color allowed quick differentiation between males and females during testing. The ordinate denotes mean time spent courting (±SEM) for a male placed with two virgins during a 10-min observation period. Each bar represents 10–15 experiments with different males. The difference between wild-type and *Ddc* flies is significant immediately after exposure to the mated females (second set of bars; $P < 0.01$).

(Fig. 3). When placed in a small chamber with receptive virgin females, naive males always courted vigorously. Previously rejected +/+ (29°C) males showed long-term depression of subsequent courtship, as previously reported. In contrast, rejected Ddc^{ts1}/*Df* (29°C) males showed no such courtship depression when subsequently tested with receptive females.

The generally normal courtship and mating sequence of Ddc^{ts1}/*Df* flies suggests that the mutations do not grossly affect overall behavior. We thought it worthwhile to examine this in more detail—specifically, to investigate several sensorimotor responses of *Drosophila* that are simple, well-characterized, and easily quantified. Positive phototaxis is the tendency of flies to run toward light when disturbed. Negative geotaxis is their similar tendency to run vertically upward. We measured both properties using the behavioral and experimental design of Benzer (28). Geotaxis of mutant Ddc^{ts1}/*Df* (29°C) flies was similar to that of wild-type flies, indicating that their strength, coordination, and walking abilities were normal. In tests of phototaxis, Ddc^{ts1}/*Df* (29°C) flies showed some deficit: a distinct subpopulation of ≈5% of the Ddc^{ts1}/*Df* (29°C) flies showed little phototaxis, although their electroretinograms (32) were normal. The other 95% of the flies phototaxed normally. (In the learning tests reported here, we used only flies preselected for normal phototaxis.)

In T-maze tests of olfactory acuity mutant Ddc^{ts1}/*Df* (29°C) flies could detect the odorants (although they avoided octanol slightly less than wild-type flies), and they could discriminate between different concentrations of these odorants (data not shown). Therefore, the ability of mutant flies to smell the odorants used in our learning tests appeared nearly normal. Their threshold for proboscis extension in response to sucrose, on the other hand, was markedly increased, from 0.004 M sucrose, characteristic of wild-type flies, to 0.025 M sucrose for Ddc^{ts1}/*Df* (29°C) flies (data not shown). Nevertheless, both genotypes responded reliably to the strong (1.0 M) sucrose stimulus used in our positive-reinforcement training.

DISCUSSION

Mutations in the *Ddc* locus interfere with learning in *Drosophila* roughly in parallel with their effect on brain L-dopa decarboxylase enzyme activity and much more than they affect other types of behavior. The fact that two different temperature-sensitive *Ddc* mutations produce flies with temperature-sensitive deficiencies in learning is the strongest evidence that the block in learning is directly due to genetic alteration of the *Ddc* locus rather than to allele-specific or genetic-background effects.

Some indirect experiments suggest that the block results from depletion of the neurotransmitter products of the dopa decarboxylase enzyme, probably dopamine or serotonin. Preliminary experiments with Ddc^{ts1}/*Df* flies indicate that the enzyme is severely depleted in flies after 24 hr at the restrictive temperature (29°C). Nevertheless, learning levels in these flies are the same ($\Lambda = 0.12$) as unshifted flies of that genotype. The severe temperature-induced decrease in learning ability comes 48 hr later, along with marked depletion of one of the transmitter products; serotonin-like immunoreactivity disappears at that time from most serotonergic cells in the central nervous system (33).

We do not know at this point whether only one of the monoamines is important for learning in *Drosophila*. In peripheral synapses in other arthropods, the most common modulatory transmitter is octopamine (1–3). We are fairly certain that octopamine is not critically involved in our *Drosophila* learning, because *Ddc* mutations, which block learning, leave octopamine synthesis unaltered, and because *per*° mutations, which produce a partial (65%) block in octopamine synthesis (18), have no effect on learning or memory in our tests (unpublished data). This leaves dopamine and serotonin, the *Ddc* metabolic products, as prime suspects. In *Aplysia*, work on the siphon withdrawal response implicates serotonin exclusively among monoamines. In *Drosophila*, at

present, there is no evidence to favor one monoamine over the other. We may acquire such evidence if we can alleviate the *Ddc* mutants' learning deficit by feeding them a transmitter's precursors, as clinicians alleviate human Parkinsonian symptoms with L-dopa.

In vertebrates, most drugs that alter monoamine metabolism also affect learning (as well as other behavioral and emotional states). The interpretation of these experiments is clouded by drug side-effects and possibly by the diversity of monoaminergic (and peptidergic) systems. Moreover, different transmitters may act in series or parallel in a single functionally relevant neural circuit. Consequently, assigning defined behavioral roles to given neurotransmitters is probably simplistic. All this said, one is left in *Drosophila* with robust phenomenology—a block in a specific monoamine-synthetic enzyme produces a roughly proportional block in associative learning with two different reinforcements, while leaving taxes and stereotyped behavior patterns relatively intact. This suggests a specific neural role for the enzyme's monoamine products in flies, with relatively little effect on other behavior. Clues to this role come from the present findings considered in conjunction with work on other animals.

In the simplest case, a neural analog of classical conditioning in *Aplysia*, serotonin, can substitute for the unconditional stimulus—the (negative) reinforcement (10). Toward the other end of the evolutionary continuum, in rats, dopamine, in the appropriate tracts and synapses, seems to be necessary for positive reinforcement (6). In *Drosophila*, at a level of neural complexity between these two animals, either dopamine, serotonin, or both are necessary for learning. Perhaps monoamines are generally involved with reinforcement. Two pieces of behavioral evidence in flies are consistent with this idea. Sucrose has to be made more concentrated (sweeter, as it were) to elicit a feeding response in *Ddc* mutants. The mutants also have to be made somewhat hungrier before they will learn reliably to respond to sucrose in an associative training situation. If one were forced to assign a simple role to monoamines in animal learning, motivation and reinforcement would come to mind. The specificity and detail of particular reinforced memories in higher organisms, and even in bees and flies, argues that at least some of the action of reinforcement has to be local (i.e., on the individual synapses that are altered). The widespread distribution of monoamines throughout the mammalian cortex is consistent with this idea.

There is another correspondence between *Drosophila* and *Aplysia* in the kinetics of learning. Experiments by Schwartz *et al.* (11) indicate that the decay of behavioral sensitization after an appropriate stimulus closely parallels the decline in cyclic AMP concentration in sensory neurons of the reflex. Furthermore, chemical agents that abbreviate the increase in cyclic AMP also shorten the duration of the behavioral response. The upshot of these experiments is that short-term memory may *be* increased cyclic AMP levels in the relevant cells in the circuit. Learning kinetics of the *Drosophila* mutants fit well with this idea. With the mutants dunce and rutabaga, which affect the kinetics of cyclic AMP metabolism (34, 35), the most striking effect is in abbreviation of short-term memory (refs. 23 and 36; T. P. Tully, personal communication). In contrast, *Ddc* mutations, which affect a metabolic step well before cyclic AMP stimulation, block the acquisition of a behavioral change with no apparent effect on memory (Fig. 2). This kinetic parallel between biochemistry and behavior in these two organisms is nice, because what Schwartz *et al.* (11) measured in *Aplysia* was memory after sensitization; what we measured in *Drosophila* was memory after associative learning.

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1. Kuba, K. (1978) *J. Physiol.* **211,** 511–570.
2. O'Shea, M. & Evans, P. D. (1979) *J. Exp. Biol.* **79,** 169–190.
3. Kravitz, E. A., Glusman, S., Harris-Warrick, R. M., Livingstone, M. S., Schwartz, T. & Goy, M. F. (1980) *J. Exp. Biol.* **89,** 159–175.
4. Kupfermann, I. (1981) *Annu. Rev. Neurosci.* **2,** 447–465.
5. Squire, L. & Davis, H. (1981) *Annu. Rev. Pharmacol. Toxicol.* **21,** 323–356.
6. Wise, R. A. (1978) *Brain Res.* **152,** 215–247.
7. Kasamatsu, T. & Pettigrew, J. D. (1979) *J. Comp. Neurol.* **185,** 139–162.
8. Daw, N. W., Rader, R. K., Robertson, T. W. & Ariel, M. (1983) *J. Neurosci.* **3,** 907–914.
9. Kandel, E. R., Brunelli, M., Byrne, J. & Castellucci, V. (1975) *Cold Spring Harbor Symp. Quant. Biol.* **40,** 465–482.
10. Kandel, E. R., Abrams, T., Bernier, L., Carew, T. J., Hawkins, R. D. & Schwartz, J. H. (1983) *Cold Spring Harbor Symp. Quant. Biol.* **48,** 821–830.
11. Schwartz, J. H., Bernier, L., Castellucci, V. F., Palazzolo, M., Saitoh, T., Stapleton, A. & Kandel, E. R. (1983) *Cold Spring Harbor Symp. Quant. Biol.* **48,** 811–820.
12. Kistler, H. B., Hawkins, R. D., Koester, J., Kandel, E. R. & Schwartz, J. H. (1983) *Soc. Neurosci. Abstr.* **9,** 915.
13. Wright, T. R. F. (1977) *Am. Zool.* **17,** 707–721.
14. Wright, T. R. F., Hodgetts, R. B. & Sherald, A. F. (1976) *Genetics* **84,** 267–285.
15. Wright, T. R. F., Bewley, G. C. & Sherald, A. F. (1976) *Genetics* **84,** 287–310.
16. Marsh, J. L. & Wright, T. R. F. (1980) *Dev. Biol.* **80,** 379–387.
17. Dewhurst, S. A., Croker, S. G., Ikeda, K. G. & McCaman, R. E. (1972) *Comp. Biochem. Physiol. B* **43,** 975–981.
18. Livingstone, M. S. & Tempel, B. L. (1983) *Nature (London)* **303,** 67–70.
19. Lindsley, D. L. & Grell, E. H. (1968) *Genetic Variations of* Drosophila melanogaster, Publ. No. 627 (Carnegie Inst., Washington, DC), p. 406.
20. Jan, L. Y. & Jan, Y.-N. (1976) *J. Physiol.* **262,** 189–214.
21. Hildebrand, J. G., Barker, D. L., Herbert, E. & Kravitz, E. A. (1971) *J. Neurobiol.* **2,** 231–246.
22. Hall, J. C., Allahiotis, S. N., Stumpf, D. A. & White, K. (1980) *Genetics* **96,** 939–965.
23. Tempel, B. L., Bonini, N., Dawson, D. & Quinn, W. G. (1983) *Proc. Natl. Acad. Sci. USA* **80,** 1482–1486.
24. Quinn, W. G., Harris, W. A. & Benzer, S. (1974) *Proc. Natl. Acad. Sci. USA* **71,** 708–712.
25. Dudai, Y., Jan, Y.-N., Byers, D., Quinn, W. G. & Benzer, S. (1976) *Proc. Natl. Acad. Sci. USA* **73,** 1684–1688.
26. Siegel, R. W. & Hall, J. C. (1979) *Proc. Natl. Acad. Sci. USA* **76,** 3430–3434.
27. Tompkins, L., Siegel, R. W., Gailey, D. A. & Hall, J. C. (1984) *Behav. Genet.* **13,** 565–578.
28. Benzer, S. (1967) *Proc. Natl. Acad. Sci. USA* **58,** 1112–1119.
29. Duerr, J. S. & Quinn, W. G. (1982) *Proc. Natl. Acad. Sci. USA* **79,** 3646–3650.
30. Lowry, O. H., Rosebrough, N. J., Farr, A. L. & Randall, R. J. (1951) *J. Biol. Chem.* **193,** 265–275.
31. Gailey, D. A., Jackson, F. R. & Siegel, R. W. (1984) *Genetics*, in press.
32. Hotta, Y. & Benzer, S. (1969) *Nature (London)* **222,** 354–356.
33. White, K. & Valles, A. M. (1984) in *Molecular Basis of Neural Development*, eds. Edelman, G. M., Gall, E. & Cowan, W. M. (Wiley & Sons, New York), in press.
34. Byers, D., Davis, R. L. & Kiger, J. A., Jr. (1981) *Nature (London)* **289,** 79–81.
35. Livingstone, M. S., Sziber, P. O. & Quinn, W. G. (1982) *Soc. Neurosci. Abstr.* **8,** 384.
36. Dudai, Y. (1983) *Proc. Natl. Acad. Sci. USA* **80,** 5445–5448.