Phototactic and Geotactic Behavior of Countercurrent Defective Mutants of *Drosophila melanogaster*

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Ten behavioral mutations, originally isolated in the countercurrent fractionation device, were tested in phototaxis and geotaxis mazes. While none of the mutations caused an altered ERG, they all caused photomaze behavior to differ from that seen in Canton-S controls. Eight of the mutants showed altered geotactic behavior. There was no correlation between the kind of change in phototactic behavior and the geomaze behavior of a given mutant. Certain mutations cause flies to be more photopositive and more geonegative than Canton-S; others result in behavior that is photo- and geopositive. The results suggest that certain mutations may be affecting visual components other than the ERG while other mutations may be more centrally or generally acting.

INTRODUCTION

Benzer (1967) devised the countercurrent apparatus to screen for behavioral mutations in *Drosophila melanogaster*. The apparatus utilizes the tendency of wild-type flies, when excited, to move rapidly toward a light source. Flies that fail, for any reason, to respond in the normal way to light are easily separated by countercurrent fractionation. If abnormal countercurrent behavior is due to a defect in either the retina or lamina of the visual

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system, it may be detected by an altered electroretinogram (ERG) (Pak, 1975). The use of the countercurrent apparatus (and other similar devices) has generated an invaluable collection of mutations used extensively in the study of the *Drosophila* visual system (see review by Pak, 1975).

A group of countercurrent defective (CCD) mutants which have normal ERGs has been recovered. The inability of these flies to behave normally in the countercurrent apparatus could be due to alterations (lesions) which affect portions of the nervous system other than what is detectable by the ERG. While no ERG differences are found, these mutations could still affect the visual system (Heisenberg and Gotz, 1975; Pak, 1975). Another possibility is that the locomotor system in some mutants could be altered, leaving the flies unable to run quickly toward a light source (Benzer, 1967). Determining the sites of action of these CCD mutants will be a complex task. We felt that it would be helpful to begin by further characterizing the behavioral effects of each mutation. We chose two behavioral classification devices, the geotaxis maze (Hirsch, 1959) and the phototaxis maze (Hadler, 1964), which are widely used in measuring geotactic and phototactic behavior in *Drosophila*. If a mutation somehow interferes with normal processes by which flies choose to go to or away from gravity or light, deviant maze behavior may be observed. Ten CCD mutants have been tested in the mazes. Their phototactic and geotactic behaviors are described below.

MATERIALS AND METHODS

Ten X-linked mutations were chosen from a larger sample of countercurrent defective mutations induced by EMS and isolated (according to the procedure found in Benzer, 1967) in the laboratory of Dr. Seymour Benzer at the California Institute of Technology. The ten mutants chosen here had all been found to have normal electroretinograms. All of the mutations are recessive and have been mapped to approximate chromosomal locations: RH41 (near *sc*), EE164 (near *sc*), RH7 (between *sc* and *v*), EE111 (near centromere), KS23 (between *sv* and *v*), YH20 (near *f*), EE85 (near centromere), TH19 (near *f*), PC16 (centromere), and KO126 (near *sc*). The mutations EE164 and RH41 are alleles as determined by countercurrent distribution; the others complement each other and thus mark separate cistrons. Recombination and complementation data will be presented elsewhere (Merriam, in preparation). The allelic pair was included here to see if different alleles of the same cistron can vary in their behavioral responses. Since the mutations described here were all isolated in the X chromosome from the Canton-S strain, the same Canton-S strain was therefore chosen as a wild-type control for all the behavioral tests. Mutant

stocks were maintained by crossing mutant males to females having Canton-S autosomes but attached X chromosomes. By crossing males from each mutant strain to the same Canton-S attached-X strain for three generations prior to testing, autosomal genetic background was randomized between the Canton-S controls and all ten mutants. The behavior of males from the mutant stocks was measured in testing devices, and wild-type males from the Canton-S attached-X strain were used as the controls.

Flies were raised on standard cornmeal–molasses–agar–yeast medium at $24 \pm 1°C$ and were tested in the behavioral devices at 4 days of age.

The countercurrent apparatus developed by Benzer (1967) was used to measure the movement to light of flies under agitated conditions. Multiple-unit mass screening geotaxis mazes (Hirsch, 1959) and phototaxis mazes (Hadler, 1964) were used to test the response of flies to gravity and light. Flies entering the mazes make a series of 15 consecutive up–down (or light–dark) choices and emerge in 16 collecting tubes at the end of the mazes. Flies making all upward (or dark) choices appear in tube 1. Flies making all downward (or light) choices appear in tube 16. Several hundred flies are tested in a maze at once and the number of flies in each collecting tube is counted and a mean geotactic or phototactic score is calculated. A geoneutral or photoneutral population would have an expected mean score of 8.5. The most geonegative (or photonegative) population would have an expected mean score of 1.0, while the most geopositive (or photopositive) population would have an expected mean of 16.0. General Electric cool white lights provided 180 ft-candles of illumination at the surface of the phototaxis maze.

RESULTS

The behavior of the ten mutants in the countercurrent apparatus is presented in Fig. 1. Photomaze and geomaze scores are shown in Table I. Scores for each of two replications are presented individually to show the level of consistency between replications. In the countercurrent apparatus Canton-S flies are strongly photopositive, but in the phototaxis maze they prefer darkness. Flies from the Canton-S wild-type strain have neutral geomaze scores which can be interpreted to mean that they have no up–down preference. The mutations vary with respect to the strength of their interference with normal countercurrent behavior and photo- and geomaze behavior. RH41 and its allele, EE164, both show only moderate impairment of countercurrent behavior. While both are photonegative in the maze, neither are as photonegative as flies from the Canton-S strain. Both of these mutations exert a rather drastic effect on geotactic behavior as evidenced by their extremely geopositive scores. The effect of RH7 on countercurrent dis-

tribution is more pronounced, and these flies are still somewhat photonega-
tive in the mazes but only slightly more geopositive than controls. The
mutation EE111 affects all three behavioral distributions in different ways.
Countercurrent behavior is definitely altered. EE111 is the only mutation
which causes flies to be strongly photopositive in the maze. Furthermore,
EE111 produces highly negative geotactic behavior. KS23 gives a greatly
modified countercurrent distribution but has less effect on performance in

Table I.   Behavior of CCD Mutants in Phototaxis and Geotaxis Mazes[a]

Mutation	Application	Photoscore $\bar{X}$ + SE (n)	Geoscore $\bar{X}$ + SE (n)
Control (CSJM)	1	4.79 ± 0.14 (448)	8.65 ± 0.25 (253)
	2	3.82 ± 0.14 (434)	8.88 ± 0.29 (226)
RH4i	1	6.38 ± 0.13 (283)[b]	15.72 ± 0.05 (204)[c]
	2	5.63 ± 0.23 (187)	15.91 ± 0.02 (208)[c]
EE164	1	6.18 ± 0.18 (239)[b]	15.99 ± 0.01 (247)[c]
	2	6.93 ± 0.17 (421)[b]	15.93 ± 0.02 (297)[c]
RH7	1	6.09 ± 0.13 (434)[b]	9.10 ± 0.20 (423)
	2	6.19 ± 0.22 (310)[b]	9.39 ± 0.18 (435)
EE111	1	11.92 ± 0.15 (398)[c]	5.15 ± 0.16 (588)[b]
	2	11.36 ± 0.17 (524)[c]	6.99 ± 0.16 (491)[b]
KS23	1	4.78 ± 0.16 (381)	6.53 ± 0.21 (427)[b]
	2	6.38 ± 0.21 (252)[b]	8.78 ± 0.26 (364)
YH20	1	8.53 ± 0.18 (251)[c]	10.99 ± 0.22 (245)[b]
	2	8.49 ± 0.18 (202)[c]	10.96 ± 0.29 (283)[b]
EE85	1	2.88 ± 0.12 (321)[b]	9.78 ± 0.21 (334)
	2	2.27 ± 0.12 (195)[b]	8.85 ± 0.20 (374)
TH19	1	5.46 ± 0.17 (217)	12.62 ± 0.34 (190)[c]
	2	6.62 ± 0.18 (230)[b]	14.18 ± 0.24 (206)[c]
PC16	1	7.33 ± 0.21 (263)[b]	4.13 ± 0.20 (233)[c]
	2	8.22 ± 0.16 (443)[c]	5.57 ± 0.27 (253)[c]
KO126	1	9.56 ± 0.15 (445)[c]	15.45 ± 0.06 (285)[c]
	2	9.55 ± 0.12 (267)[c]	15.48 ± 0.13 (237)[c]

[a] Phototactic and geotactic scores of mutant male flies were compared to pooled scores of
Canton-S control males using the nonparametric Kruskal-Wallis H test (Woolf, 1968). A χ^2
table was used to determine probability values.
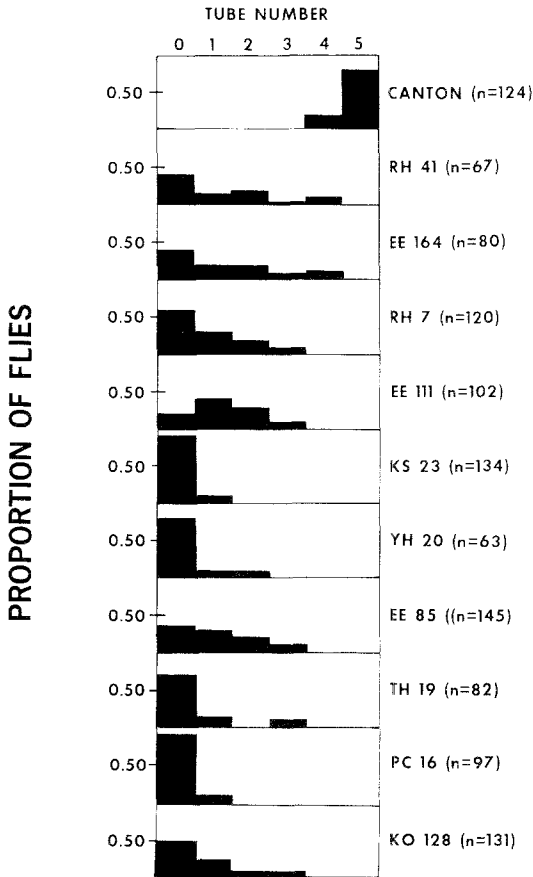[b] $p < 0.01$.
[c] $p < 0.001$.

Fig. 1. Countercurrent distributions of Canton-S and mutant flies. Tube number shows number of times flies moved toward light.

either maze. The countercurrent distribution for YH20 is similar to that shown by KS23, but in the mazes flies are completely photoneutral and somewhat geopositive. EE85 does not alter countercurrent behavior as much as some of the other mutants, nor does it seem to affect geotactic behavior. However, EE85 flies are even more photonegative than Canton-S. The effect of TH19 on countercurrent behavior is striking and behavior in both mazes is altered. PC16 and KO126 are both strong CCD mutations. PC16 tends to nearly eliminate the photonegative maze behavior seen in controls and, surprisingly, causes flies to become extremely geonegative. KO126 causes photopositive maze scores and extremely geopositive scores.

DISCUSSION

Each of the three testing devices described above measures a different behavioral trait of *Drosophila*. Two of these, countercurrent behavior and photomaze behavior, measure the response of flies to light. When flies are tested in the countercurrent apparatus, they are shaken down after passage from one tube to another. Therefore, the response of agitated flies is being measured. By contrast, flies walk undisturbed through the photomazes, selecting either the light or dark arm of the Y-tube. Lewontin (1959) showed that *D. pseudoobscura* are highly photopositive, that is, they move quickly toward light when disturbed, but when unagitated they are photonegative. Rockwell *et al.* (1975) verified this finding for *D. pseudoobscura* and *D. persimilis*. Our present results with Canton-S, which go quickly to light in the countercurrent but prefer darkness under the conditions in the maze, show the same to be true of *D. melanogaster* and suggest that two different behaviors are being evaluated by these two testing devices. However, since both measure a response to light, both behaviors are therefore dependent on the function of the visual system. Mutations which alter the ERG are known to reduce the degree of photonegativity in the maze as compared to Canton-S controls (Merriam and Markow, 1977). In the most extreme case, e.g., mutations of the no receptor potential gene (norp A) (Pak, 1975), photomaze behavior is completely photoneutral as it is with any strain tested in total darkness, suggesting the importance of the peripheral visual components in photomaze behavior. Markow and Scavarda (1976), using white-eyed *D. melanogaster,* showed that visual acuity is also important for normal behavior in the phototaxis maze. All three behaviors are dependent on an intact locomotor system: each device requires flies to move in relation to some stimulus (light or gravity). Geotaxis, as measured in the mazes, is a highly complex trait. Any factors influencing the weight, activity levels, or tendency of flies to stumble may alter geotactic behavior as measured in the maze as well as countercurrent distribution.

We are interested in whether photo- and geomaze behavior can be varied independently of each other or of phototaxis as measured in the countercurrent apparatus. Because the original search for countercurrent defective mutants was inaugurated to obtain mutants with visual defects, it is reasonable to ask if lines with additional visual defects can be identified. The fact that we chose for examination the so-called CCD mutants, those with normal ERGs, means that such defects, if any, would lie in a part of the visual system not contributing to the ERG. Since the lines tested here have been well controlled to carry the same genetic background as Canton-S, any significant deviation from the photonegativity of Canton-S can be

attributed to a loss of visual function. However, we recognize that mutant defects in other parts of the nervous system might also affect photoscores. For example, any defect which tends to randomize carrying out choices in decision making would cause a tendency towards photoneutrality.

Can we conclude that any of these ten mutations causes a partial (or complete) loss of visual function? Our strategy is to assume that lines with different scores from controls on both the photo- and geomazes do not carry a genetic defect in visual perception. (The possible objection that a line may carry two separate mutations such that the line may show alterations in both behaviors is discussed more fully below.) We note in passing that the countercurrent phenotype of each of these mutations seems to map to restricted portions of the X chromosome, making unlikely the possibility that the original CCD phenotypes are synthesized by the interactions of mutations at several loci. Thus candidate lines for visual dysfunction would be those with reduced phototatic but unaltered geotactic behavior.

Two of the lines examined here, RH7 and EE85, show significant changes in phototactic scores from the Canton-S controls but no real change in the geotactic scores. RH7 appears to involve the partial loss of vision, since that score is intermediate between the control photonegativity and photoneutrality. While those flies are photonegative, they are not as photonegative as Canton-S. RH7 might represent the kind of lesion of the peripheral visual system discussed by Heisenberg and Gotz (1975) that produces normal ERGs. Further work is necessary to classify this mutation by a set of standard tests representative of the fly's total repertoire of visual and other sensory responses. EE85 is less easy to understand since it causes flies to be more photonegative than Canton-S. In fact, the photomaze scores of EE85 are the most photonegative reported for this genus (Dobzhansky and Spassky, 1967; Woolf, 1972; Polivanov, 1975; Markow, 1975). One possible hypothesis to explain this result is that the mutant might remove some inhibitory effect, possibly from other sensory inputs, that normally decreases the full weight of the photonegative tendency.

The rest of the mutations mostly affect both photomaze and geomaze behavior to varying degrees. Only flies bearing the KS23 mutation show little or no change from both control maze scores. From this we may conclude that phototaxis measured by the countercurrent distribution method may be causally separated from phototaxis measured in Hirsch-Hadler mazes. Among the other mutations, KO126 and especially EE111 are the only two which cause photopositive behavior in the maze. It is interesting that EE111 causes flies to be quite geonegative, while KO126 gives extremely geopositive behavior. The other mutations give photoscores which tend to be somewhere between photoneutrality (8.5) and the photonegativity shown by Canton-S. If the Canton-S genetic background,

which is taken to be representative of an unaltered nervous system, causes flies to be photonegative in the maze, each mutation appears to reduce the degree of that photonegativity. The fact that many of these same mutations also exert an extreme effect on geotaxis implies that they might operate on a nervous system process or structure central (possibly in the brain) to all three behaviors.

It is possible that the differences in geotactic or phototactic behavior seen in flies from mutant stocks is due to loci in the X chromosome other than the CCD loci. The X chromosomes carrying the CCD mutation could vary at other loci which influence behavior in the geotaxis and phototaxis mazes, possibly from the action of the EMS. However, our data tend to exclude this possibility. Evidence that the altered geotactic and phototactic scores of CCD flies are due to the CCD mutations themselves comes from comparing effects of noncomplementing mutants. For example, RH41 and EE164 are mutations to the same locus (see Materials and Methods) induced during separate mutagenesis projects, but they are effectively identical in their effects on all three behaviors reported here. In preliminary experiments, the same was found for other loci as well.

While the mutations described here affect either photomaze behavior alone or both phototaxis and geotaxis, further testing might reveal other CCD mutations which alter geotaxis only. Experiments designed to assess the behavior of visually (ERG) defective flies in the geotaxis maze are also needed. The results reported here serve to further define and distinguish the effects of particular mutations on behavior. Expanding our behavioral characterizations of CCD mutants may help determine if a lesion caused by a particular mutation affects a particular sense organ or is more generally or centrally acting. Adequate behavioral descriptions will become especially meaningful when a given mutant gene is linked to a biochemical function.

ACKNOWLEDGMENTS

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