Proc. Natl. Acad. Sci. USA
Vol. 87, pp. 8632–8636, November 1990
Genetics

A genetic study of the anesthetic response: Mutants of *Drosophila melanogaster* altered in sensitivity to halothane

(general anesthesia/neurogenetics/"inebriometer")

K. S. KRISHNAN* AND HOWARD A. NASH

Laboratory of Molecular Biology, National Institute of Mental Health, Bethesda, MD 20892

Communicated by Dan L. Lindsley, August 3, 1990

ABSTRACT **In an attempt to identify genes that control or encode the targets of general anesthetics, we have chemically mutagenized fruit flies and selected four lines that show an abnormal response to the volatile anesthetic halothane. Specifically, about 2-fold higher concentrations of halothane are required to induce the loss of motor control in the mutant flies. Fine mapping of two isolates indicates that they alter a previously uncharacterized gene of *Drosophila*. In the absence of anesthetics, these mutants display alterations of behavior that imply changes in the adult and the larval neuromuscular system.**

The mechanism by which anesthetics cause their typical response—loss of consciousness and insensitivity to pain—remains speculative (1, 2). Many plausible subcellular targets for anesthetics have been proposed (3–8) but it has been difficult to choose among these candidates since each is supported by evidence of modification by anesthetics. Similarly, when one seeks to understand the chemical basis of anesthetic action, it is hard to choose between theories that call for the direct effect of anesthetics on a receptor or channel and theories in which the function of the putative target is altered only as a secondary consequence of an anesthetic-induced alteration in the lipid portion of cell membranes (9–11). One useful resource to help distinguish between proposed mechanisms for anesthesia would be a collection of mutants that were each altered in their responsiveness to anesthetics; the collection would be particularly useful if each mutant affected only a single genetic locus. The ability to isolate such mutants would imply not only that the components are under genetic control but also that there are only a limited number of ways that anesthetics induce their effects. Moreover, among such mutants should be some that are altered in the abundance or properties of the anesthetic target(s). These mutants might therefore provide useful material for biochemical tests of particular theories of anesthesia. In addition, molecular genetic techniques might permit the identification of a component that is required for anesthesia even in the absence of an *a priori* hypothesis. It should also be pointed out that such a mutant collection might well identify interesting and novel components of the nervous system even if their involvement in the anesthetic response is only indirect.

In this work we have tested the feasibility of obtaining mutants of *Drosophila melanogaster* that change its response to anesthetics. *Drosophila* has been long known to be reversibly anesthetized—i.e., rendered motionless and unresponsive to stimuli—by the classical anesthetic diethyl ether (12). Fruit flies are also sensitive to the nonflammable volatile anesthetic halothane ($CF_3CHClBr$) and we used this agent in our initial experiments. We have restricted our genetic study to the induction phase of anesthesia—i.e., the process of losing consciousness. In this way we tried to concentrate on the neural response to halothane and bias against alterations that affect only the metabolism or distribution of the drug (which we reasoned might be more prominent among mutants affected in the recovery from an anesthetic dose). Moreover, we have not attempted to isolate sensitive mutants but have limited our screen to mutants resistant to halothane. This was done in the hope of avoiding genetic changes that simply weaken the fly and thereby make it nonspecifically sensitive to an insult. To simplify the labor involved in the search for mutants, we used well-established procedures (13, 14) for crossing males that had been fed a chemical mutagen with females whose X chromosomes are attached. This allowed us to examine the offspring *en masse* not only for dominant mutations but for recessive X chromosome-linked mutations that were transmitted directly from fathers to sons. From about 20,000 chemically mutagenized flies we have identified four resistant mutants and herein report their initial characterization.

MATERIALS AND METHODS

Fly Stocks and Genetic Methods. The wild-type strain OreR was obtained from R. Greenspan (Roche Institute of Molecular Biology, Nutley, NJ). The attached-X stock *C(1)DX y f* and the X chromosome balancer *M5* were from S. Haynes (National Institute of Child Health and Human Development). The mapping stock *y* w^a *ct g f* balanced with *FM7a* was from J. Kennison (National Institute of Child Health and Human Development). *Df(1)RK2*, *Df(1)RK5*, and *Df(1)rD17* were from the laboratory of B. Ganetzky (University of Wisconsin). All other stocks were from the Bloomington Stock Center (Bloomington, IN).

Flies were grown in cornmeal molasses agar at 22°C. Chemical mutagenesis with ethyl methanesulfonate was performed as described by Lewis and Bacher (13); treated OreR males were mated to attached-X females for 1–2 days and the F_1 generation was screened for anesthesia resistance. The resistant alleles so identified have been maintained for >1 year as hemizygous lines with attached-X females, as homozygous lines, and as heterozygous lines with the *M5* balancer; with routine precautions, the phenotype of each line has remained stable.

Behavioral Assays. Column fractionation of flies was performed in an "inebriometer", which was constructed essentially as described (15). For screening mutagenized populations, 500–1000 flies were loaded in one run and exposed to halothane (Halocarbon Laboratories, North Augusta, SC). The anesthetic was delivered (6 liters/min) using a Penlon evaporator and compressed air; concentration was set at 0.5% and checked with a Riken model 18 gas indicator. After 30–45 min, the flies remaining in the column were collected

The publication costs of this article were defrayed in part by page charge payment. This article must therefore be hereby marked "*advertisement*" in accordance with 18 U.S.C. §1734 solely to indicate this fact.

*On leave from Tata Institute of Fundamental Research, Bombay.

by flushing the column with 100% CO_2. For anesthesia testing of individual lines, flies were between 1 and 4 days old posteclosion; they were sorted under CO_2 anesthesia and kept for 1 day on fresh medium prior to testing. Typically, 20–100 flies of a particular genotype were loaded onto the column, usually together with an equal or larger number of flies that were morphologically distinct, and exposed to halothane as above; flies were collected every 2 min.

For visual inspection of the response to anesthetics, a "separator" chamber was used. This device consists of a 200-ml egg-shaped glass enclosure (made from a separator flask) that can be connected to a cylindrical glass chamber below it. Groups of 10–12 flies were introduced into the upper chamber and, after connecting the two components, the system was perfused with anesthetic at a flow rate of 6 liters/min. Flies were observed in the lower chamber with the help of a hand lens; those that were lying on their backs on the base of this chamber were counted as anesthetized.

The motor activity of adult flies was assessed with a "monitor". The device consists of a borosilicate tube (diameter = 13 mm; length = 75 mm) fitted with two photodetectors and two infrared photodiodes. About 10 flies were introduced into the tube, which was then placed in a dark chamber. Movement of the flies creates a signal in the photodiode; the number of such spikes that accumulate in a standard time interval (usually 60 sec) was printed as the output of a microprocessor. Typically, a group of 10 flies produced 250–300 spikes per min. The chamber was perfused at 1 liter/min with anesthetic and the time required to have three consecutive intervals with no spikes was noted.

RESULTS

Isolation of Halothane-Resistant (*har*) Mutants. We reasoned that because anesthetics may interact with critical components of the nervous system it might not be possible to obtain viable flies that have radical alterations in anesthetic sensitivity. Accordingly, we screened mutagenized stocks of flies with an apparatus designed to detect subtle differences in fly behavior. An inebriometer, designed by Weber (15) to assess alcohol resistance in natural populations of flies, consists of a glass column containing about 20 nylon mesh baffles on which flies can rest. When exposed to an anesthetic such as halothane, flies tend to fall off the baffles but have repeated opportunities to settle on a baffle that is lower in the column. We screened for flies that eluted from the column at late times, thereby enriching for flies that had not suffered the loss of motor control that typifies anesthesia. After cycling again through the inebriometer, candidate male flies were used to establish separate lines with attached-X females. In all, we screened about 20,000 mutagenized flies, tested about 100 individual lines, and recovered four mutants. Fig. 1 presents inebriometer elution profiles of wild-type flies and the four halothane-resistant mutant lines (*har* mutants). Although the graph of Fig. 1 shows results from a single experiment for each line, several repetitions yielded comparable results. As shown in Table 1, on average the mutants are 1.5 (*har56*) to 7.9 (*har38*) less responsive than control flies in this test.

Table 1. Average response of mutants in the inebriometer

Strain	Response index	*n*
Wild-type	0.82 ± 0.14	21
har56	0.55 ± 0.11	9
har63	0.43 ± 0.09	5
har85	0.16 ± 0.12	21
har38	0.10 ± 0.07	18

The proportion of flies eluted from the column after 30 min of exposure to 0.5% halothane is shown. The data are averages (±SD) of *n* experiments.

To observe the behavior of the mutants more closely, we exposed groups of about 10 male flies of each line to different concentrations of halothane in a glass enclosure (separator) that contained no baffles. To measure the anesthetic response we simply scored how many flies were lying motionless on their backs at the bottom of the vessel after a given time (Fig. 2). The results support the interpretation of the inebriometer tests; mutant lines appear to be more resistant than the wild-type line in their response to halothane. This is especially clear at 1.0% and 1.5% halothane, where each mutant line is significantly different (95% by Scheffe's F test) from OreR. From the shift in the concentration of halothane required to render half of the flies anesthetized in 5 min, it appears that the mutants are 1.5–2.5 times as resistant as their wild-type counterparts.

Genetic Characterization of the Mutants. When mutant male flies are maintained as stocks with nonresistant females whose X chromosomes are attached, all male progeny and no female progeny show halothane resistance. This indicates that each of the *har* mutations maps to the X chromosome. To determine the behavior of the *har* alleles in the presence of another X chromosome, we crossed mutant males to normal females and established homozygous and heterozygous stocks. Females homozygous for three *har* mutants are readily distinguished from wild-type flies (Table 2). How-

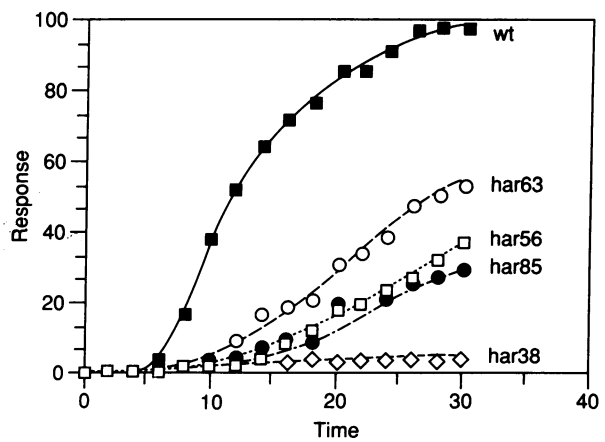

FIG. 1. Performance of wild-type (wt) and mutant male flies in the inebriometer assay. The percentage of flies that are recovered from the bottom of the apparatus is plotted against time of exposure (min) to 0.5% halothane. The wild-type strain used for comparison is OreR.

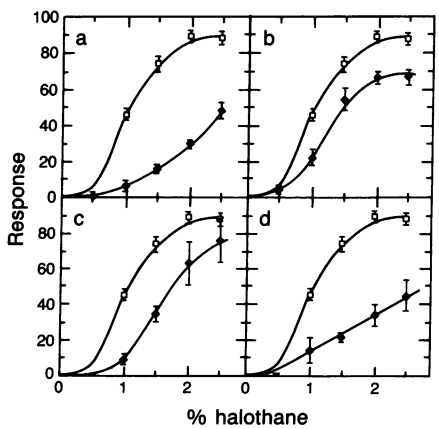

FIG. 2. Performance of wild-type and mutant male flies in the separator assay. The proportion of flies anesthetized (±SEM) after 5 min of exposure to the indicated concentrations of halothane is shown. □, OreR (har$^+$); ◆, mutants. (*a*) *har38*. (*b*) *har56*. (*c*) *har63*. (*d*) *har85*.

Table 2. Anesthesia response of female flies

Strain	Response index	*n*
+/+	0.77 ± 0.17	6
38/38	0.24 ± 0.10	15
63/63	0.32 ± 0.12	7
85/85	0.32 ± 0.14	15
56/56	0.65 ± 0.18	13
38/+	0.64 ± 0.08	6
63/+	0.41 ± 0.05	12
85/+	0.63 ± 0.15	7

About 10 females of the indicated *har* genotype were exposed to 2.0% halothane in the separator assay. The fraction (±SD) of flies (from *n* experiments) that are scored as anesthetized after 5 min is reported. The wild-type X chromosome in these experiments was the *M5* balancer.

ever, *har56* homozygotes cannot be reliably distinguished from wild-type flies in this test. To determine the dominant or recessive character of the remaining mutants, heterozygotes containing an X chromosome from a *har* strain and a wild-type X chromosome were tested in the separator assay. According to these tests (Table 2), *har63* is a dominant mutation (and will be henceforth referred to as *Har63*), whereas *har38* and *har85* are recessive.

Because *har38* and *har85* show the most resistance to anesthesia, and are therefore easiest to study, we undertook the genetic mapping of these alleles. We first located the *har85* allele by meiotic recombination with several easily scored markers—*w*, *ct*, *g*, and *f*—that divide the X chromosome into roughly equal intervals. Tests of anesthesia resistance in recombinant lines indicated that *har85* maps to the *g-f* interval. All of 12 lines that had inherited this segment from the nonresistant parent but had a crossover in the *w-ct* or *ct-g* intervals were sensitive to halothane. However, 2 of 6 lines that had a crossover in the *g-f* interval displayed resistance.

Table 3. Deficiency mapping of *har* mutants

Deficiency	Cytology	Wild type	*har38*	*har85*
dm75e19	3C11;3E4	0.69 ± 0.28	0.66 ± 0.16	0.58 ± 0.12
HC244	3E8;4F11-12	0.36 ± 0.14	0.44 ± 0.34	0.55 ± 0.18
HF366	3E7-8;5A7	0.73 ± 0.11	0.71 ± 0.17	0.57 ± 0.19
RC40	4B1;4F1	0.25 ± 0.12	0.27 ± 0.12	0.17 ± 0.11
JC70	4C15-16;5A1-2	0.39 ± 0.20	0.31 ± 0.15	0.34 ± 0.20
C149	5A8-9;5C5-6	0.44 ± 0.09	0.53 ± 0.10	0.40 ± 0.15
N73	5C2;5D5-6	0.55 ± 0.23	0.40 ± 0.15	0.46 ± 0.10
HA32	6E4-5;7A6	0.48 ± 0.12	0.43 ± 0.24	0.34 ± 0.16
C128	7D1;7D5-6	0.38 ± 0.19	0.41 ± 0.25	0.42 ± 0.17
RA2	7D10;8A4-5	0.74 ± 0.14	0.71 ± 0.17	0.70 ± 0.17
KA14	7F1-2;8C6	0.65 ± 0.12	0.48 ± 0.12	0.58 ± 0.14
C52	8E;9C-D	0.60 ± 0.27	0.65 ± 0.26	0.52 ± 0.14
v-L15	9B1-2;10A1-2	0.60 ± 0.18	0.36 ± 0.19	0.37 ± 0.12
N110	9B3-4;9D1-2	0.46 ± 0.18	0.36 ± 0.19	0.43 ± 0.16
HC133	9B9-10;9E-F	0.61 ± 0.10	0.55 ± 0.14	0.67 ± 0.17
ras-v-Cc8	9D1-2;10A2-3	0.75 ± 0.05	0.65 ± 0.15	0.64 ± 0.25
RA37	10A6;10B15-17	0.53 ± 0.09	0.52 ± 0.07	0.47 ± 0.13
HA85	10C1-2;11HA1-2	0.58 ± 0.13	0.47 ± 0.28	0.58 ± 0.10
KA6	10E1;11A7-8	0.72 ± 0.15	0.78 ± 0.09	0.65 ± 0.16
RA47	10F1;10F9-10	0.39 ± 0.05	0.28 ± 0.28	0.25 ± 0.20
N105	10F7;11D1	0.78 ± 0.08	0.54 ± 0.12	0.56 ± 0.08
KA10	HA1;11A7-8	0.37 ± 0.10	0.55 ± 0.13	0.56 ± 0.10
JA26	11A1;11D-E	0.57 ± 0.06	0.61 ± 0.20	0.76 ± 0.15
HF368	11A2;11B9	0.44 ± 0.27	0.42 ± 0.15	0.54 ± 0.26
C246	11D-E;12A1-2	0.57 ± 0.38	0.45 ± 0.20	0.51 ± 0.19
N12	11D1-2;11F1-2	0.30 ± 0.04	ND	0.33 ± 0.16
HA92	12A6-7;12D3	0.61 ± 0.16	0.54 ± 0.15	0.65 ± 0.12
KA9	**12E2-3;12F5-13A1**	**0.43 ± 0.15**	**0.10 ± 0.13**	**0.11 ± 0.07**
RK2	**12D2-E1;13A2-5**	**0.88 ± 0.05**	**0.33 ± 0.12**	**0.36 ± 0.16**
RK5	12E9-11;13A9-B1	0.50 ± 0.11	0.58 ± 0.08	0.68 ± 0.12
r-D17	14F6;15A6	0.79 ± 0.12	0.68 ± 0.15	0.81 ± 0.13
N19	17A1;18A2	0.36 ± 0.18	0.56 ± 0.11	0.21 ± 0.09
E128	17C;18A	0.54 ± 0.15	0.41 ± 0.14	0.41 ± 0.18
JA27	18A5;20A	0.65 ± 0.13	0.54 ± 0.20	0.63 ± 0.16
HF396	18E1-2;20	0.64 ± 0.25	0.74 ± 0.05	0.77 ± 0.18
16-3-22	19D1;20A2	0.61 ± 0.16	0.63 ± 0.15	0.64 ± 0.25
B57	19E1-2;19F1	0.69 ± 0.17	0.70 ± 0.19	0.73 ± 0.16
Q539	19E6;19F6-20A1	0.72 ± 0.24	0.62 ± 0.13	0.63 ± 0.17
DCB1-35b	19F1-2;20E-F	0.64 ± 0.08	0.78 ± 0.10	0.79 ± 0.09
JC4	20A1;20E-F	0.47 ± 0.18	0.63 ± 0.19	0.39 ± 0.19

Female flies bearing one X chromosome from OreR or the indicated *har* mutant and an X chromosome from the indicated deficiency strain were constructed; estimates of the cytological extent of each deficiency were provided by the supplier. Groups of 6–20 heterozygous females were tested for anesthesia resistance in the separator; the average response (±SD) is shown. Since the strain background of each deficiency influences the response to anesthetics, one should read the table horizontally and not vertically. The two deficiencies that produce a highly significant increase in resistance (99% by Scheffe's F test) when heterozygous with *har38* or *har85* are printed in boldface type. ND, not determined.

A precise map position for *har85* and *har38* was established by complementation mapping. We crossed these mutants to a set of stocks bearing deficiencies of the X chromosome and we scored the anesthesia phenotype of the resulting heterozygous females. Table 3 shows that both alleles display halothane resistance when placed in trans to either of two deficiencies, *Df(1)KA9* or *Df(1)RK2*. The effect is specific: no other deficiency tested produces anesthesia resistance in heterozygotes. The behavior of heterozygotes of *har38* and *har85* bearing deficiencies *KA9*, *RK2*, and *Df(1)RK5* was confirmed by duplicate tests with the inebriometer (not shown). Fig. 3 summarizes the mapping experiments from this region; the data indicate that *har38* and *har85* alleles map to the same small segment of the X chromosome. As expected from the recombination mapping of *har85*, this segment lies in the interval between the markers *g* and *f*. To test for allelism of *har38* and *har85*, females carrying one X chromosome from each mutant strain were constructed and tested as in Table 2. These heterozygotes showed partial complementation, yielding values for the fraction of flies anesthetized after 5 min of 0.47 ± 0.12. These results suggest that *har38* and *har85* are the same locus or belong to loci that interact with one another genetically to produce anesthesia resistance. We have maintained stocks that contain a *har38* or *har85* chromosome plus a *KA9* or *RK2* chromosome for >10 generations. Such stocks are genetically stable; specifically, all the progeny are halothane resistant. This indicates that the *har38* and *har85* lines are each affected in only a single locus. If there were a second locus that contributes to the phenotype of *har38* or *har85*, recombinants that had acquired the wild-type copy of this putative locus from the deficiency parent should not show anesthesia resistance.

Behavioral and Morphological Characterization of the Mutant Strains. It is possible that mutants isolated as resistant to anesthetics would be altered primarily in the metabolism of these compounds; such pharmacokinetic mutants might have little value in understanding the neural response to anesthetics. However, two of our mutants, *har38* and *har85*, display abnormal behavior in the absence of anesthetics. The most obvious phenotype is that these mutants produce considerably fewer offspring than wild-type flies. The defective production of offspring is particularly noticeable when the mutants are raised in competition with other strains; however, homozygous stocks are reasonably vigorous and can be maintained without special handling. Adults of these strains also have a subtle change in morphology: the abdomens of these flies appear to be more slender and elongated than wild-type flies. We do not know if this alteration represents a developmental defect or reflects altered physiology. Most interestingly, the flies perform poorly in a standard countercurrent geotaxis assay (14). This is largely because they tend to walk by fits and starts, taking a few steps and then stopping before starting again. Mechanical shock such as banging enhances the hesitancy of these flies; however, given a long enough time they display a pronounced negative geotaxis. All of the above traits—low viability, hesitant walking, and abdominal morphology—map to the same deficiencies that uncover the anesthesia resistance behavior. Larvae of *har38* and *har85* also show unusual behavior in the absence of anesthetics. In the phototaxis assay of Lilly and Carlson (16), third instar larvae of these mutants migrate less markedly from well-lit sectors to dark sectors in the time allotted (response index of 0.19 compared to 0.68 for wild-type larvae). The larvae are clearly mobile; we do not know if their phototaxis defect represents failure to sense light, sluggish mobility, or indifference to illumination. Taken together, our data convince us that the phenotype of mutants *har38* and *har85* does not depend solely on a pharmacological challenge. These flies have suffered alterations in physiology, presumably reflecting changes in the neuromuscular system, that render them atypical in their response to anesthetics.

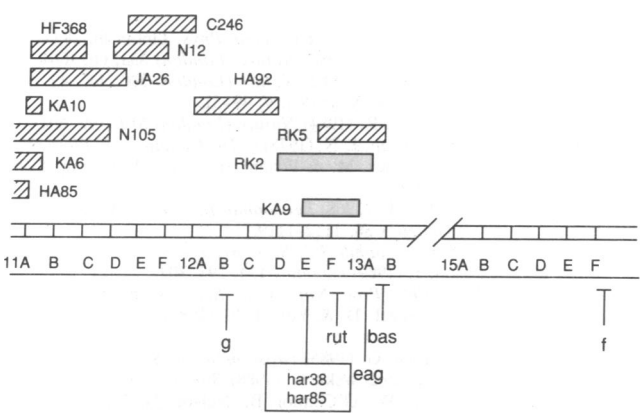

FIG. 3. Cytological mapping of *har38* and *har85*. The extent of several deficiencies is indicated by a bar above a diagram (not to scale) of cytological positions along the X chromosome. Those deficiencies that uncover the resistance phenotype of *har38* and *har85* are shaded grey and those that do not are hatched. Positions of the morphological marker alleles garnet and forked (*g* and *f*) as well as the nearby neurological mutants rutabaga (*rut*), ether-a-go-go (*eag*), and bang sensitive (*bas*) are indicated at the bottom. The map position of *rut* is taken from the literature (17, 18). The map position of *eag* is based on the observation by B. Ganetzky and colleagues (cited in ref. 19) that *eag* is uncovered by deficiencies *RK2* and *RK5* but not by *KA9*; similarly, the map position of *bas* is based on the observation by T. Homyk (personal communication) that *bas* is uncovered by *RK5* but not by *KA9*.

The way in which these mutants respond to anesthetics provides some clue as to the nature of the change in the nervous system. When exposed to anesthetics, individuals of the *har38* and *har85* strains tend to become immobile. This has been quantitated with an activity monitor. On exposure to 1.5% halothane, wild-type flies take 4.9 ± 1.6 min to cease activity, whereas *har38* and *har85* become inactive after only 2.0 ± 1.1 min and 1.8 ± 0.7 min, respectively. When assaying the anesthetic response in the separator test, we have noted that these same mutants stand as if frozen for a considerable period before falling over and thus being counted as anesthetized. Taken together, it appears that mutations *har38* and *har85* do not make flies uniformly insensitive to halothane but provoke a unique response, freezing, to this agent. It is possible that freezing represents the activation by halothane of a behavioral pattern that is normally turned off in flies. Alternatively, freezing could indicate that the mutant flies are sensitive to the hypnotic actions of halothane but are resistant to its effects on motor control. In any case, the freezing response probably accounts for the mutants' performance in the inebriometer. In contrast, mutants *har56* and *Har63* do not show a freezing behavior; they simply take longer to respond to the anesthetic. For example, in tests of activity, these mutants require 6.5 ± 2.0 min and 6.8 ± 1.8 min, respectively, to become quiet in response to 1.5% halothane. In the absence of anesthetics mutants *har56* and *Har63* do not show obvious defects in phototaxis, geotaxis, longevity, or morphology. Homozygous *Har63* females are sterile but we have not yet ascertained whether this property is caused by the same genetic alteration that leads to anesthetic resistance.

DISCUSSION

In this work we report the isolation of mutants of *Drosophila* that are altered in their response to halothane. We believe these mutants can be of general value in exploring the mechanism of anesthesia because the response of the fruit fly to anesthetics resembles that of higher organisms. We have used our separator assay to assess the effect of six other

clinically useful volatile anesthetics; not only do all succeed in anesthetizing the flies but also the relative potency of these compounds agrees well with published values for anesthesia in humans (K.S.K., D. B. Campbell, and H.A.N., unpublished observations). It should be emphasized that the anesthetic effects in *Drosophila* are reversible. We have kept flies asleep with 1% halothane for 16 hr; if one takes precaution against dehydration, at least half of these flies recover after removal of the anesthetic. Another feature of anesthesia in flies that is reminiscent of the behavior in higher organisms is the progressive loss of neural functions with increasing dose of the anesthetic agent (20). In summary, despite differences in the anatomy of their respective nervous systems, the chemistry and physiology of anesthesia in flies closely parallel that observed in vertebrates.

Although limited, our current set of mutants provides some interesting leads. We have tested our mutants with six additional anesthetics and we find that each mutant shows cross-resistance to some but not all of these agents (K.S.K., D. B. Campbell, and H.A.N., unpublished observations). Thus, our mutants are not halothane specific. Other evidence makes it clear that at least mutants *har38* and *har85* change the fly's behavior in the absence of anesthetics. Adults of these strains have an altered pattern of walking and larvae show defects in light-evoked movement. These phenotypes point to an alteration in the nervous system that presumably underlies their altered response to anesthetics. Although all of our mutants were selected as resistant to anesthetics, we suspect that the tendency of *har38* and *har85* to become immobile in response to halothane implies that their nervous system is actually sensitive to this agent but responds to it in an unorthodox way. Nevertheless, it should be emphasized that the mutants *har38* and *har85* define at least one gene that has not been previously characterized as a locus affecting behavior in *Drosophila*. The deficiency mapping of Table 3 and Fig. 3 locates the gene(s) of these alleles to the 12E1–E9 region. Although several previously described neurological mutants map nearby, the complementation pattern of these mutants with deficiencies in the region is clearly different from that observed with *har38* and *har85*. Our experience with these two mutants convinces us that mutations isolated as halothane resistant in the inebriometer, in addition to their utility in exploring the basis of anesthesia, may also be valuable for uncovering genetic elements of the nervous system.

There have been previous efforts to use genetic methods to gain insight into the mechanism of anesthetics. For example, colonies of mice have been bred selectively for an altered response to nitrous oxide (21). However, the genetic complexity underlying the phenotype of the resistant and sensitive colonies made it difficult to pursue genetic studies. An organism with much more tractable genetics, the roundworm *Caenorhabditis elegans*, has also been examined for the anesthetic response (22). Here, single locus mutants were identified that render *C. elegans* abnormally sensitive to some but not all anesthetics (23, 24). It is too early to speculate on the relationship between the *C. elegans* mutants and those that we have obtained in *D. melanogaster*. However, we note that the nervous system of *Drosophila* contains tens of thousands of neurons, whereas *C. elegans* has only 302 nerve cells. Accordingly, we suspect that in *Drosophila*, anesthetics may have the opportunity to depress the kind of subtle targets that are found in more complex nervous systems of higher organisms. In keeping with this idea, it should be pointed out that much higher concentrations of halothane (ED_{50} = 3.2%) are required to render *C. elegans* immobile (22) than are required to anesthetize *D. melanogaster* (ED_{50} ≈1.0%; see Fig. 2).

Most previous work with *Drosophila* genetics and anesthesia has involved diethyl ether. The mutants Shaker, Hyperkinetic, and ether-a-go-go display violent motion upon etherization (25) and various ether-sensitive strains that die after exposure have been described (26, 27). In addition, Eth29, a strain resistant to the lethal effects of high concentrations of ether, has been described (28); this strain was found to be resistant to the induction of anesthesia by several agents (29). It will be of interest to compare these strains with those that we have isolated. One obvious difference is that Eth29, apparently a natural isolate and not the result of a mutagenesis, contains multiple changes that map to different chromosomes. Royden (30) has found that technical knockout (*tko*) wakes from ether anesthesia more promptly than do wild-type flies. This mutation, which has a bang-sensitive phenotype, maps near the white locus (31) and therefore represents a gene not included among mutants that we have mapped. In general, we believe that it will be most fruitful to study the effects of all of these mutations in combination with the *har* mutants described in this work. Evidence for suppression or enhancement of the halothane-resistance phenotype will help to arrange these genes as participants in one or more pathways affected by anesthetics.

We are grateful to Ralph Greenspan and Ken Weber for encouragement and advice at the earliest stages of this project and to John Carlson for his comments on this manuscript. We also thank John Whitaker and Gail Ginsburg for expert technical assistance and the technical development section of the National Institute of Mental Health for construction of much of the apparatus used in this work.

1. Ueda, I. & Kamaya, H. (1984) *Anesth. Analg. (Cleveland)* **63**, 929–945.
2. Albrecht, R. F. & Miletich, D. J. (1988) *Gen. Pharmacol.* **19**, 339–346.
3. Miller, K. W., Braswell, L. M., Firestone, L. L., Dodson, B. A. & Forman, S. A. (1986) in *Molecular and Cellular Mechanisms of Anesthetics*, eds. Roth, S. H. & Miller, K. W. (Plenum, New York), pp. 125–137.
4. Moody, E. J., Suzdak, P. D., Paul, S. M. & Skolnick, P. (1988) *J. Neurochem.* **51**, 1386–1393.
5. Segal, I. S., Vickery, R. G., Walton, J. K., Doze, V. A. & Maze, M. (1988) *Anesthesiology* **69**, 818–823.
6. Ori, C., Ford-Rice, F. & London, E. D. (1989) *Anesthesiology* **70**, 541–544.
7. Bangham, A. D. & Hill, M. W. (1986) *Chem. Phys. Lipids* **40**, 189–205.
8. Franks, N. P. & Lieb, W. R. (1988) *Nature (London)* **333**, 662–664.
9. Franks, N. P. & Lieb, W. R. (1982) *Nature (London)* **300**, 487–493.
10. Miller, K. W. (1986) *Trends NeuroSci.* **9**, 49–51.
11. Franks, N. P. & Lieb, W. R. (1984) *Nature (London)* **310**, 599–601.
12. Ashburner, M. & Thompson, J. N. (1978) in *The Genetics and Biology of Drosophila*, eds. Ashburner, M. & Wright, T. R. F. (Academic, London), Vol. 2a, pp. 1–109.
13. Lewis, E. B. & Bacher, F. (1968) *Drosophila Inf. Serv.* **43**, 193.
14. Benzer, S. (1967) *Genetics* **58**, 1112–1119.
15. Weber, K. E. (1988) *Drosophila Inf. Serv.* **67**, 91–93.
16. Lilly, M. & Carlson, J. (1989) *Genetics* **124**, 293–302.
17. Livingstone, M. S. (1985) *Proc. Natl. Acad. Sci. USA* **82**, 5992–5996.
18. Dudai, Y., Sher, B., Segal, D. & Yovell, Y. (1985) *J. Neurogenet.* **2**, 365–380.
19. Lindsley, D. L. & Zimm, G. (1985) *Drosophila Inf. Serv.* **62**, 103.
20. Kirschfeld, K. & Baier-Rogowski, V. (1988) *Biol. Cybern.* **58**, 1–11.
21. Koblin, D. D., Lurz, F. W., O'Connor, B., Nelson, N. T., Eger, E. I., II, & Bainton, C. R. (1984) *Anesth. Analg. (Cleveland)* **63**, 35–39.
22. Morgan, P. G. & Cascorbi, H. F. (1985) *Anesthesiology* **62**, 738–744.
23. Sedensky, M. & Meneely, P. M. (1987) *Science* **236**, 952–954.
24. Morgan, P. G., Sedensky, M. M., Meneely, P. M. & Cascorbi, H. F. (1988) *Anesthesiology* **69**, 246–251.
25. Kaplan, W. D. (1969) *Genetics* **61**, 399–409.
26. Kidd, K. K. (1963) *Drosophila Inf. Serv.* **37**, 49.
27. Peterson, P. A. (1963) *Drosophila Inf. Serv.* **21**, 88.
28. Ogaki, M. & Nakashima-Tanaka, E. (1967) *Jpn. J. Genet.* **42**, 387–394.
29. Gamo, S., Ogaki, M. & Nakashima-Tanaka, E. (1981) *Anesthesiology* **54**, 289–293.
30. Royden, C. S. (1987) Ph.D. thesis (Univ. of California, San Francisco).
31. Royden, C. S., Pirrotta, V. & Jan, L. Y. (1987) *Cell* **51**, 165–173.