A Genetic and Mosaic Analysis of a Locus Involved in the Anesthesia Response of *Drosophila melanogaster*

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Manuscript received February 6, 1997
Accepted for publication June 26, 1997

ABSTRACT

We describe a genetic and behavioral analysis of several alleles of *har38*, a mutant with altered sensitivity to the general anesthetic halothane. We obtained a *P*-element-induced allele of *har38* and generated several excision alleles by remobilizing the *P* element. The mutants *narrow abdomen* (*na*) and *har85* are confirmed to be allelic to *har38*. Besides a decreased sensitivity to halothane, all mutant alleles of this locus cause a characteristic walking behavior in the absence of anesthetics. We have quantified this behavior using a geotaxis apparatus. Responses of the mutant alleles to different inhalational anesthetics were tested. The results strongly favor a multipathway model for the onset of anesthesia. Mosaic flies were tested for their response to halothane and checked for their abnormal walking behavior. The analysis suggests that both the behaviors are exhibited only by such mosaics as have the entire head of mutant origin. It is likely that this focus represents an element of a common pathway in the anesthetic response to several inhalational anesthetics but not all. This result is the first demonstration of regional specificity in the CNS of any animal for general anesthetic action.

GENERAL anesthetics comprise a diverse class of chemical compounds, from elemental gases like nitrogen and argon to complex halocarbons like halothane. They cause a state of reversible unconsciousness in humans and other animals. How these volatile anesthetics act has been the subject of intense speculation for well over a hundred years. The Meyer-Overton rule postulates that the potency of a general anesthetic is directly related to its lipid/water partition coefficient (MEYER 1899; OVERTON 1901). Thus, for all anesthetics, the same number of lipid-associated molecules elicits the same level of response. This rule has stood its ground in the face of a host of newly discovered anesthetics. In its simplest interpretation, the Meyer-Overton rule implies that all anesthetics first dissolve in neural plasma membranes and then act on a common cellular substrate. This interpretation is embodied in the "unitary target hypothesis" according to which all general anesthetics act by the same cellular mechanism (GILMAN *et al.* 1991). A large body of biophysical and medical experimentation has supported the idea that general anesthetics act primarily by dissolving in lipid membranes. However, an understanding of molecular mechanisms and cellular substrates of anesthesia continues to be a challenge.

Genetics has proven to be an ideal tool in the study of complex processes that involve the response of the animal as a whole. Although in its infancy, genetic analysis of the anesthetic response has already provided unique contributions to the study of mechanisms of anesthetic action (MIR and KRISHNAN 1995). Mutants showing altered response to anesthetics have been obtained in *Caenorhabditis elegans* and in *Drosophila melanogaster* (SEDENSKY and MENEELY 1987; KRISHNAN and NASH 1990; MORGAN and SEDENSKY 1994; LEIBOVITCH *et al.* 1995). These studies argue against a nonspecific action of anesthetics and hint at the possibility of more than one target or pathway for general anesthetic action. An inconvenient aspect of the *C. elegans* studies is that the state of anesthesia in this animal is poorly defined. The worms take up to several hours to show the full response, and the required anesthetic concentrations are very high compared to those of other animal models used in anesthesiology (SEDENSKY and MENEELY 1987; MIR and KRISHNAN 1995). On the other hand, in Drosophila, the other model organism for genetic studies of the nervous system, characteristics of response to anesthetics are very similar to those of higher animals (KRISHNAN and NASH 1990; MIR and KRISHNAN 1995).

We have earlier described the identification of single gene mutations in Drosophila that cause altered response to the anesthetic effects of halothane (KRISHNAN and NASH 1990). The four mutants we isolated, *har38*, *har56*, *Har63* and *har85* (har for halothane anesthesia resistance), show differential resistance to various inhalational anesthetics. While *har38* and *har85* mutants show resistance to halothane, methoxyflurane, chloroform and trichloroethylene, their responses to diethyl ether, isoflurane and enflurane are comparable to wild

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type. On the other hand, while *Har63* is similar to *har38* in response to halothane and methoxyflurane, the two mutants differ dramatically in their responses to chloroform, trichlorethylene and enflurane. Yet another spectrum of sensitivities is displayed by *har56* (NASH *et al.* 1991; CAMPBELL and NASH 1994).

Here we report the first characterization of multiple mutant alleles of an anesthesia response locus in Drosophila previously defined by the mutation *har38* (renamed na^{har38}). We chose na^{har38} for investigation as these mutants have exceptionally strong and completely recessive phenotypes. In addition to their previously described halothane-resistant behavior, na^{har38} mutants show two additional phenotypes that are genetically inseparable. First, they show a peculiar saccadic walking behavior: they take a few steps, stop abruptly, and then start again. Second, they have abnormally elongated and cylindrical abdomens. All three phenotypes are shown to varying degrees by the new mutants described in this study. The abnormal walking behavior shown by *na* mutants shows that genes involved in the anesthesia response play additional roles in normal motor control in the wild-type animal. Although mutations in different genes result in differing responses to distinct anesthetics, EMS-, X-ray- and transposon-induced alleles of *na* result in a very similar spectrum of behavioral responses to a variety of general anesthetics. This suggests that distinct pathways exist for the onset of general anesthesia.

Mutants affected in anesthesia response could be affected in any of three general functions: (1) the delivery of anesthetic to the target, (2) the reception of the anesthetic signal, and (3) molecular or cellular elements that transduce the anesthetic signal into behavioral unconsciousness. To try to distinguish among these possibilities, we performed blastoderm fate mapping experiments to determine the focus for the na^{har38} phenotype. Our results show that all behavioral phenotypes of na^{har38} result from the action of the mutation in the cephalic ganglion. A major question in anesthesiology relates to a possible aesthesis center, which might be the target of anesthetic action. A formal possibility that the focus, defined by the behavior of mosaic mutants, is itself the aesthesis center is discussed.

MATERIALS AND METHODS

Fly stocks and culture: Flies were grown at 22° on standard corn meal agar. Fly stocks, *na* (*narrow abdomen*) and *Horka* (with the genotype *+/Y; +/+; mwh Horka e/TM3 Sb* and *+/+; +/+; T(1;3) OR60/TM3 Sb*), were obtained from the Drosophila Stock Center at Bloomington, Indiana. The *na* mutation maps to the same location as *har38* and results in an anatomical phenotype similar to that caused by *har38*. It was isolated in 1934 (MILLER 1934). Other than its anatomical features no specific phenotype or characterization is available. X-ray-induced deficiencies *Df(1) CO_1 (12C6;12E5)* and *Df(1) CO_2 (12AB;12E3-E6)*, and the *P*-element-containing line *GR871* were obtained from CHARLES OH (University of California, Berkeley). Other strains used in the experiments were from the TIFR stock collection. Halothane was obtained from Industrial Solvents, India as well as from Anaquest. Enflurane and isoflurane were obtained from Ohmeda Pharmaceutical Products Inc., NJ. Ether, chloroform and trichloroethylene were analytical grade reagents from Glaxo India Ltd., Bombay.

Behavioral assays: *Anesthetic resistance:* Flies were grown at 22° and collected 24–36 hr after eclosion and tested for anesthetic resistance as described previously (KRISHNAN and NASH 1990). We used a device called an "inebriometer," which consists of a 5-foot cylindrical glass column of ~3 inches inner diameter. The column is interrupted by 20 hemicone-shaped baffles of nylon mesh (Figure 1). Anesthetics at specified concentrations in air were passed through the column with the aid of a standard hospital kettle (Fluotec-Mark 3, Ohmeda Pharmaceutical Products Inc., New Jersey). The hospital kettle is essentially a large heat-capacity metal chamber in which the anesthetic liquid is allowed to evaporate at room temperature. Air flowing through the vaporizing chamber carries the anesthetic vapor and this is mixed with pure air at a defined proportion. The proportions are preset for halothane by the manufacturer and are controlled by a marked knob to obtain the desired concentration. A Riken model 18 gas meter was used to measure anesthetic concentrations. The concentrations were calculated using the tables provided by the manufacturers. For convenience, all flows were adjusted to obtain a reading of 0.50% on the gas meter. This corresponded to 0.50% for halothane and 0.40% for trichloroethylene. Anesthetics enflurane, chloroform and isoflurane were used at 0.54, 0.52 and 0.52%, respectively.

The experimental paradigm is as follows. The column is equilibrated with a given concentration of anesthetic for 10 min. Flies are loaded at the top of the column and a constant anesthetic concentration is confirmed using the Riken gas meter. Flies that are anesthetized tumble down through the multiple baffles ("elute") and are collected in a vial kept at the bottom of the column. Vials are replaced every 2 min. Anesthetic flow is stopped after 30 min and remaining flies are eluted from the column by exposing them briefly to carbon dioxide. The "anesthesia response index" is calculated as the ratio of flies eluted by anesthetics in the first 30 min to the total number of flies. In the case of wild-type flies this index is nearly 1.00, and an index of zero indicates complete resistance. In the case of excisions and their various combinations, where the background was ry^{506}, the anesthetic was stopped after 20 min and response was calculated as the ratio of flies eluted in the first 20 min to the total number of flies. Before switching to a different anesthetic, the kettle was cleaned three times with acetone and thoroughly dried in a stream of air. The cleaning was continued until the Riken gas meter read zero, on a maximal setting of the concentration-dial on the kettle.

Walking behavior: We measured geotaxis using a counter current apparatus similar to the one originally described by Benzer (1967). The apparatus consisted of six tubes 9.2 cm long and 2.4 cm in diameter. Approximately 25 flies were loaded into the bottom of the first tube. Flies were then allowed to walk against gravity for 7 sec after which the tubes were shuffled. The flies that have reached the top of the first tube are now ready to walk up the second tube, while the flies that have not reached the top are knocked back to the bottom. The process is repeated giving each fly five chances to cross from one tube to the other. Wild-type flies in this interval of time always manage to go to the other side of the tube array. At the end of the assay, all the wild-type flies were found in the last tube. In the case of na^{har38} almost all of the flies remained in the first tube. Weaker alleles resulted in

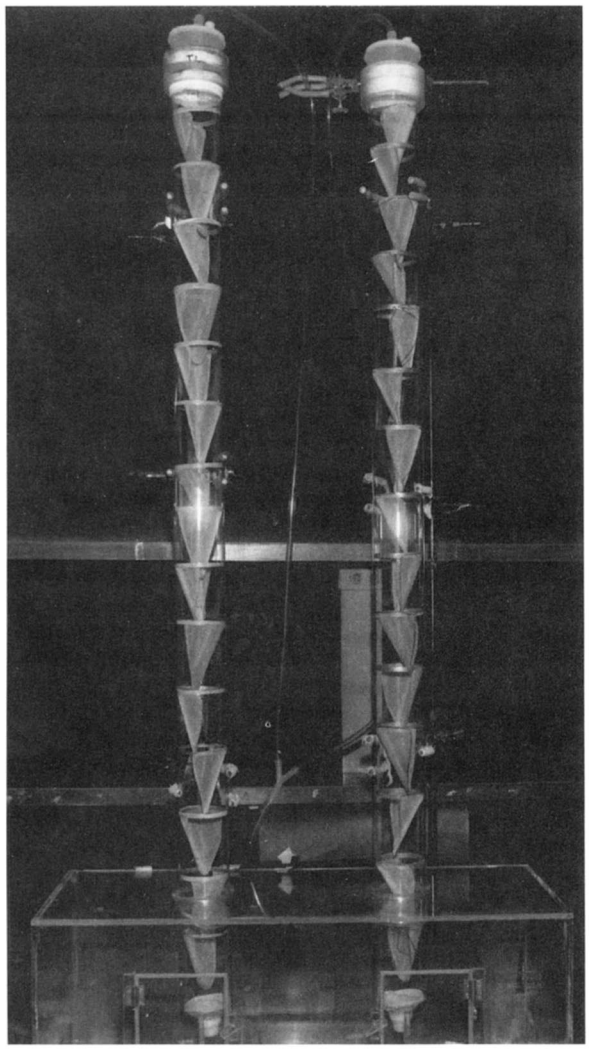

FIGURE 1.—The "inebriometer" used for quantitative analysis of anesthetic behavior. The column is a modified version of the one originally designed by KEITH WEBER (WEBER 1988). Two hundred to 300 flies are loaded into the space on top of the column that has been previously equilibrated with the desired anesthetic concentration. Flies are eluted with the same concentration of anesthetic for 30 min and are collected as they fall through the baffles in vials kept at the bottom of the column. Flies remaining in the column at the end of the run are eluted with a flow of CO_2.

some dispersion. Most of the flies remained in the first tube but a few reached further tubes. Geotaxis response index was calculated as $\Sigma\ (T-1)n/5N$ where T is the tube number, n the number of flies in a particular tube and N is the total number of flies. The summation is over values of T ranging from one to six. Thus, an index of 0.0 results from all the flies staying in tube one and an index of 1.0 corresponds to all flies reaching tube number six. Intermediate values reflect different dispersion patterns.

***P*-element-induced mutagenesis:** We had earlier mapped the na^{har38} mutation to polytene bands 12E2-E11. To obtain *P*-element insertions into *na*, we mobilized a *P* element *GR871* at 12E-F and screened progeny for failure to complement the na^{har38} phenotype. In brief, the crosses were as follows. *P*(ry^+) *GR871*/Y; +/+; *Sb* ($\Delta 2\text{-}3\ ry^+$) ry^-/ry^- males were picked and crossed to na^{har38}/na^{har38}; +/+; ry^-/ry^- virgins. From the progeny, P^*/na^{har38}/; +/+; ry^-/ry^- virgin females were picked and tested for anesthetic resistance in the inebriometer. Virgin females that stayed back in the inebriometer after 30 min exposure to 0.5% halothane were mated to na^{har38}/Y; +/+; ry^{506}/ry^{506}. Lines were set up and tested again in homozygous condition and also heterozygous with na^{har38}. From nearly 30,000 P^*/na^{har38}; +/+; ry^-/ry^- animals, we set up lines from about a thousand potential resistant females and finally selected nine lines that failed to complement the resistance of na^{har38} to halothane in the inebriometer assay. A line designated as *P890* was selected for further analysis because of its robust and completely recessive behavior. *P890/P890* flies are viable and show a measurable resistance to halothane. Deficiency mapping and complementation analysis confirmed it to be an allele of *na*. This insertional allele also causes the characteristic walking behavior of na^{har38} mutant.

***In situ* hybridization:** Procedures used for chromosomal *in situs* were as previously described (PALLANCK *et al.* 1995). In brief, wandering third instar larvae grown at 18° were used to obtain salivary glands, which were "squashed" onto glass slides to provide spread out polytene chromosomes. A fragment of *P*-element DNA labeled with biotinylated dUTP was used to probe polytene chromosomes and streptavidin-HRP complex was used to visualize hybridization. After the peroxidase reaction, chromosomes were stained with 5% Giemsa for 3 min and observed under phase contrast.

Excision analysis: The *P* element from the *P890* chromosome was mobilized using a transposase source, and *X*-chromosomal lines from which the transposon had been excised were isolated and analyzed. In brief, *P890*(ry^+)/*FM7*; +/+; ry^-/ry virgins were crossed to +/*Y*; +/+; *Sb*$^-$ ($\Delta 2\text{-}3\ ry^+$) ry^-/*Ubx* ($\Delta 2\text{-}3\ ry^+$) ry^- males. The jump starter males from the progeny bearing the genotype *P890*(ry^+)/*Y*; +/+; *Sb*$^-$ ($\Delta 2\text{-}3\ ry^+$) ry^-/ry^- were crossed to *FM7a*/*FM7a*; +/+; ry^-/ry^- virgins, and +/*FM7*; +/+; $ry^-/Sb^+\ ry^-$ females were picked and lines were set up with *FM7*/*Y*; +/+; ry^-/ry^- males. The appropriate progeny were test crossed to either na^{har38} and tested as transheterozygote females or males, for both halothane resistance and walking behavior.

Generation of mosaics: External cuticular markers *yellow* (*y*) and *white apricot* (w^a) were genetically recombined onto the chromosome bearing na^{har38} mutation. To do this, we crossed the homozygous na^{har38} virgins to marked *X*-chromosome-bearing males. F_1 female progeny of this cross were further crossed to *FM7* males and the F_2 population was tested for halothane resistance. Males marked *y* and w^a, which showed anesthetic resistance, were selected and lines established. These were further tested and finally a true recombinant was selected. *y* w^a na^{har38}/*y* w^a na^{har38} or *y* w^a na^{har38}/*FM7*; +/+; +/+ virgins were crossed to *Horka* males bearing the genotype +/*Y*; +/+; *Horka e*/*TM3*, and in the F_1 potential female progeny were scored for mosaics (SZABAD *et al.* 1995). Mosaic flies were mixed with a large number of identifiable na^{har38} and wild-type flies before loading on the inebriometer. The external cuticular markers on eluted flies were scored after each run. For each class of mosaic, data were pooled and behavior indices calculated as previously described.

To confirm that external cuticular markers were good indicators for the genotype of underlying tissue, we directly examined this issue in ~30 mosaic animals that we recovered. An *X*-chromosome P(ry^+ *lac-Z*) line *ETX* 28 (ANAND *et al.* 1990) showing ubiquitous β-galactosidase expression in the brain and in thoracic muscles provided an internal marker for brain and thoracic tissue. *P-ry*$^+$ *ETX28*/*Y*; +/+; *Horka e*/*TM3*, which had internally marked *X* chromosome, were crossed to *y* w^a na^{har38} virgins to generate identifiable mosaic progeny. The external cuticular markers (yellow cuticle and white apricot eyes) were scored for the extent of mutant tissue and the internal marker was followed by staining frozen sections for

β-galactosidase. In all cases, there was an excellent correspondence between the β-galactosidase expression in internal tissues and the absence of yellow cuticle (or white eyes) in the overlying ectoderm.

RESULTS

Phenotypes of na^{har38}: *The anesthetic response:* We have renamed the mutant *har38* as na^{har38} to indicate its designation as an allele of the *na* locus. The index of response to halothane calculated from inebriometer elution profiles of na^{har38} is compared to that for wild-type flies in Table 1. The indices reflect a strong resistance to halothane-induced anesthesia in the mutants. In 30 min, during which the flies are continuously exposed to halothane in the inebriometer, all the wild-type flies are anesthetized whereas almost all the na^{har38} flies withstand this lengthy anesthetic exposure. The response index for wild type (*CS* as well as *Oregon-R*) is nearly unity. The na^{har38} mutant flies thus seem to show an almost complete resistance to halothane. This phenotype is completely recessive and is uncovered by deficiencies $Df(1)RK_2$, $Df(1)CO_1$ and $Df(1)CO_2$. The complementation data with these deficiencies narrows down the chromosomal location of na^{har38} to 12E2-E5 region from 12E2-E11 reported earlier (KRISHNAN and NASH 1990).

Saccadic walking behavior of na^{har38}: We observed a peculiar walking behavior of na^{har38} flies; their walk was interrupted by pauses. We calculated that these pauses led to relatively longer run times in a geotaxis apparatus. We found that in our counter current apparatus if the time interval was kept to 7 sec, na^{har38} flies failed to cross from one tube to another and therefore remained confined to the first tube. However, all the wild-type flies crossed from one tube to the other in this interval. The geotaxis response calculated as a summed index is shown in Table 1. The na^{har38} mutants showed a reduced response indicative of their inability to cross the first tube in the interval of time. The heterozygotes of na^{har38} with the three deficiencies $Df(1)CO_1$, $Df(1)CO_2$ and $Df(1)RK_2$ showed an identical response. The response index for both *CS* and *Oregon-R* wild-type flies is close to unity. Heterozygotes of na^{har38} with deficiencies in regions away from this locus exhibit behavior indistinguishable from wild type (data not shown). This phenotype, like the halothane resistance, was completely recessive. It is important to observe that this geotaxis response index does not indicate a slow constant climbing velocity for na^{har38} flies but reflects the frequent halts made during the saccadian walking pattern of na^{har38} mutants. This observation suggests that gene products required for anesthesia response play additional important roles in nervous system function, specifically in motor control pathways.

The anatomical mutant phenotype of na^{har38}: We noticed that the na^{har38} mutants had abdomens that were more cylindrical and narrower than wild type. Although a clear and distinguishing phenotype in na^{har38} flies (Figure 2), this phenotype was not as pronounced in flies carrying other alleles. This phenotype maps to *na* as evidenced by subsequent experiments described in this report. All of these features caused by na^{har38}, *i.e.*, anesthetic resistance, walking behavior and narrow abdomen were uncovered by the same set of deficiencies.

TABLE 1

Halothane and geotactic response of wild-type and na^{har38} flies

Genotype	Halothane response	Geotactic response
CS/Y	0.89 ± 0.09	0.93 ± 0.02
Oregon R/Y	0.85 ± 0.07	0.89 ± 0.03
na^{har38}/Y	0.10 ± 0.06	0.0 ± 0.0
CS/CS	0.85 ± 0.09	0.89 ± 0.01
Oregon R/Oregon R	0.81 ± 0.06	0.88 ± 0.02
na^{har38}/na^{har38}	0.04 ± 0.03	0.0 ± 0.0
na^{har38}/CS	0.85 ± 0.08	0.87 ± 0.01
$CS/Df(1)CO_1$	0.84 ± 0.08	0.87 ± 0.01
$na^{har38}/Df(1)CO_1$	0.14 ± 0.04	0.0 ± 0.0
$CS/Df(1)CO_2$	0.86 ± 0.09	0.85 ± 0.01
$na^{har38}/Df(1)CO_2$	0.20 ± 0.06	0.01 ± 0.01
$CS/Df(1)RK_2$	0.88 ± 0.07	0.89 ± 0.02
$na^{har38}/Df(1)RK_2$	0.18 ± 0.08	0.01 ± 0.01

CO_1 = Df(1)12C6; 12E5; CO_2 = Df(1)12AB; 12E3-E6; RK_2 = Df(1)12D2-E1; 13A2-A5.

New alleles of na^{har38}: *narrow abdomen: na* mutants showed an anesthetic response index of 0.47 ± 0.17 for halothane, significantly lower than wild type. This anesthesia-resistant phenotype suggested to us that it may be an allele of na^{har38}. We confirmed allelism to na^{har38} by complementation tests that showed that the narrow abdomen, anesthetic resistant and walking phenotypes were not complemented by *na*. This result has also been confirmed by D. B. CAMPBELL and H. A. NASH (personal communication). Interestingly *na* mutants show somewhat weaker anesthetic resistance and geotactic (walking) defects than na^{har38}. However, the complementation data (Table 3) and the additional observation that *na* phenotypes are uncovered by the same set of deficiencies that uncover na^{har38} unequivocally prove that na^{har38} allelic to *na*. Although *na* was discovered quite sometime ago (MILLER 1934), the only description available is scanty. However in keeping with the tradition of Drosophila nomenclature, the mutant *har38* will be referred to as na^{har38}.

har85: The *har85* mutant (now designated as na^{har85}) was isolated in the same screen as na^{har38} for halothane anesthesia resistance (KRISHNAN and NASH 1990). It has an identical spectrum of resistance to different volatile anesthetics as na^{har38} (NASH *et al.* 1991; CAMPBELL and NASH 1994). It also shows the saccadic walking behavior in the absence of exposure to anesthetics and abdominal morphology like na^{har38}. Our earlier studies indicated na^{har85} may be allelic to na^{har38} (KRISHNAN and

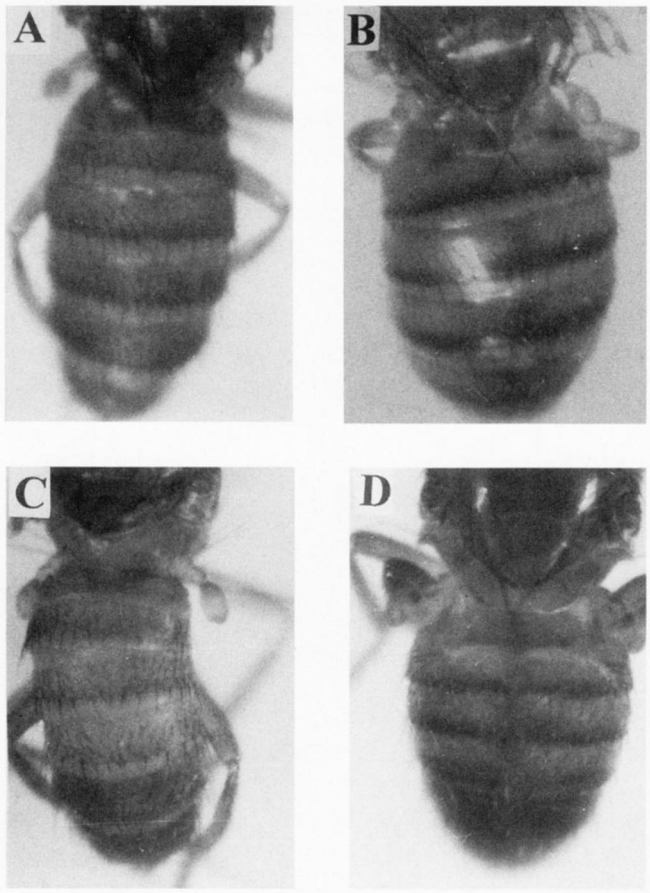

FIGURE 2.—The abdominal phenotype of na^{har38}. B and D show the wild-type abdomen (female and male), while A and C are abdomens of na^{har38} female and male flies, respectively.

NASH 1990). Our suggestion, however, was tentative and based only on two deficiencies that uncovered the phenotype associated with both these mutations. The results presented in Tables 3 and 4 are more conclusive in this regard and show that na^{har85} fails to complement na^{har38} and all *na* alleles for both halothane resistance and geotactic response phenotypes.

P-allele (na^{P890}): To obtain insertional alleles of *na*, we mobilized a *P* element (GR871) inserted at 12E-F, close to *na*. Chromosomes bearing the *P* element at potentially new locations were screened for their inability to complement na^{har38}. Halothane-resistant females heterozygous for na^{har38} were selected and single female lines set up. Of these, nine lines bred true and were stable for several generations. The line designated *P890* showed strong resistance in trans with na^{har38} and itself showed appreciable resistance in homozygous and hemizygous conditions. The presence of a single *P* element at 12EF position in this line was confirmed by *in situ* hybridization. This line was then selected for further characterization. The behavior of na^{P890} is shown in Table 2. When hemizygous, na^{P890} flies show a weaker halothane resistance index compared to na^{har38}. The transheterozygote of na^{har38} and na^{P890} shows an intermediate response. na^{P890} also has a strong geotaxis phenotype and the geotaxis index is close to that for na^{har38}.

Excisions: To confirm that the halothane-resistant phenotype of na^{P890} was caused by a *P* insertion into *na,* we remobilized the *P* element in na^{P890} flies and looked to revert the anesthesia-resistant phenotype. We screened a total of 100 *P*-excision lines for complementation of na^{har38}. Of these hundred, a set of 17 lines showing varying degrees of halothane resistance when hemizygous were characterized. We chose four lines for more detailed analysis. The two lines designated as na^{Pex3} and na^{Pex4} behaved like wild type and showed very little halothane resistance, whereas two other lines, na^{Pex1} and na^{Pex2}, showed a considerable resistance. Even though these excision alleles do not cause resistance comparable to that caused by na^{P890}, the phenotypes are stronger in flies heterozygous for either excision allele and na^{har38} or other *na* alleles. The excision lines na^{Pex1} and na^{Pex2} showed the saccadic walking reflected in a poor geotaxis response like the na^{har38} mutant. This phenotype when manifested in the excision lines was again not as pronounced as that caused by na^{har38} and other *na* alleles. The phenotypes of the excision lines were uncovered by the same set of deficiencies that uncovered the *na* behavior. na^{Pex1} and na^{Pex2} therefore represent excision alleles. On the other hand, na^{Pex3} and na^{Pex4} behave like precise excisions. Both the halothane and geotactic response of na^{Pex3} and na^{Pex4} lines are comparable to wild type in all combinations. All the alleles resulted in similar phenotypes and failed to complement the same set of deficiencies. Thus the mutants represent an allelic series with respect to halothane resistance and walking behavior: $na^{har38} > na^{har85} > na^{P890} > na > na^{Pex1} > na^{Pex2}$.

Response of *na* alleles to different anesthetics: The availability of multiple mutant alleles of an anesthesia-response locus allowed us to address an important question in anesthesiology: are there distinct pathways to general anesthesia? To address this question, we tested all *na* alleles for their response to several different anesthetics. The responses shown in Figure 3 suggest that *na* mutations specifically affect a pathway to general anesthesia induced by a subset of anesthetics. All *na*

TABLE 2

Halothane and geotactic response of na^{P890} in comparison to wild-type and na^{har38} flies

Genotype	Halothane response	Geotactic response
$CS/Y; ry^{506}/ry^{506}$	0.93 ± 0.07	0.89 ± 0.04
$CS/CS; ry^{506}/ry^{506}$	0.90 ± 0.06	0.88 ± 0.03
$na^{har38}/Y; ry^{506}/ry^{506}$	0.08 ± 0.04	0.0 ± 0.0
$na^{har38}/na^{har38}; ry^{506}/ry^{506}$	0.06 ± 0.04	0.0 ± 0.0
$na^{P890}/Y; ry^{506}/ry^{506}$	0.29 ± 0.12	0.06 ± 0.01
$na^{P890}/na^{P890}; ry^{506}/ry^{506}$	0.31 ± 0.13	0.06 ± 0.02
$na^{P890}/na^{har38}; ry^{506}/ry^{506}$	0.14 ± 0.09	0.03 ± 0.01

mutants without exception are indistinguishable from wild type in their responsiveness to enflurane and isoflurane. However, they show different degrees of resistance to anesthesia by halothane and chloroform. The alleles na^{har38}, na^{Pex1} and na^{Pex2} alone show appreciable resistance to trichloroethylene-induced anesthesia. The small differences in the response index between *na* and other strains in the case of enflurane and isoflurane, although significant by statistical tests, were in the range of variations due to background differences between strains. This was also the case for the response of some *na* mutants to trichloroethylene. This conclusion was arrived at after plotting a frequency distribution of the range of response indices, which was bimodal. Excision alleles na^{Pex1} and na^{Pex2}, which caused weaker responses to halothane and chloroform, caused resistance to trichloroethylene comparable to that caused by na^{har38}. Two excisions, na^{Pex3} and na^{Pex4}, probably precise, resulted in responses indistinguishable from wild type for all the anesthetics. The profile of resistance follows the order $na^{har38} > na^{har85} > na^{P890} > na > na^{Pex1} > na^{Pex2}$ in the case of halothane. The resistance to chloroform follows a very similar pattern. In the case of trichloroethylene na^{Pex1}, na^{Pex2} and na^{har38} alone caused appreciable resistance. The salient feature of this analysis is the normal response to enflurane and isoflurane exhibited by all *na* mutants.

Mosaic analysis: The complex anatomical, motor and anesthesia phenotype of *na* mutants made it especially important for us to study the anatomical focus for the anesthesia-resistance phenotype. For instance, we specifically wished to know whether narrow abdomen inhibited delivery of anesthetics to their target site. To investigate this issue we generated mosaic animals. Mosaic patches of mutant tissue were produced in an essentially wild-type background by *Horka* (SZABAD *et al.* 1995), a third chromosome dominant mutation that results in the preferential loss of paternal chromosomes. Loss of the paternal *X* chromosome gives rise to male tissue in heterozygous female background. The na^{har38} mutation in a marked *X* chromosome is derived from the maternal parent and therefore the mutant patch is easily identified by the external markers and by change in structures like sex comb, wings and genitalia. Since the chromosome loss occurs predominantly in early cell divisions the mosaic patches are large and contiguous. A total of 132 mosaics were obtained after scoring nearly 20,000 potential flies. Of these mosaics, 102 were obtained starting with the allele na^{har38} and 30 such mosaics were derived from the allele na^{har85}. The paternal *X* chromosome was marked with the lacZ reporter gene in a few mosaics; however in most cases the paternal *X* chromosome was wild type. The mosaics so generated were tested for both halothane resistance and walking behavior along with other flies that included both anesthetic-resistant and normal flies to act as internal positive and negative controls. The pattern

TABLE 3

Halothane response of flies bearing different alleles and their various combinations

	na^{har38}	na^{har85}	na^{P890}	*na*	na^{Pex1}	na^{Pex2}	CO_1	CO_2	RK_2	*CS*	na^{Pex3}	na^{Pex4}
na^{Pex4}	0.86 ± 0.07	0.86 ± 0.07	0.83 ± 0.08	0.84 ± 0.07	0.85 ± 0.08	0.89 ± 0.08	0.87 ± 0.08	0.83 ± 0.05	0.85 ± 0.08	0.86 ± 0.09	0.85 ± 0.07	0.84 ± 0.08
na^{Pex3}	0.86 ± 0.08	0.87 ± 0.06	0.85 ± 0.07	0.85 ± 0.07	0.84 ± 0.06	0.90 ± 0.08	0.84 ± 0.06	0.85 ± 0.06	0.86 ± 0.07	0.89 ± 0.10	0.85 ± 0.08	
CS	0.85 ± 0.08	0.82 ± 0.09	0.80 ± 0.10	0.83 ± 0.10	0.83 ± 0.09	0.82 ± 0.08	0.81 ± 0.10	0.86 ± 0.09	0.88 ± 0.08	0.85 ± 0.09		
RK_2	0.18 ± 0.08	0.27 ± 0.08	0.32 ± 0.13	0.50 ± 0.12	0.49 ± 0.11	0.58 ± 0.09						
CO_2	0.20 ± 0.06	0.26 ± 0.07	0.35 ± 0.09	0.43 ± 0.14	0.50 ± 0.10	0.57 ± 0.09						
CO_1	0.14 ± 0.04	0.16 ± 0.06	0.25 ± 0.17	0.40 ± 0.10	0.39 ± 0.05	0.49 ± 0.04						
na^{Pex2}	0.22 ± 0.04	0.20 ± 0.09	0.25 ± 0.09	0.34 ± 0.10	0.51 ± 0.10	0.54 ± 0.05						
Na^{Pex1}	0.16 ± 0.06	0.19 ± 0.08	0.24 ± 0.06	0.21 ± 0.09	0.48 ± 0.10							
na	0.23 ± 0.11	0.13 ± 0.10	0.23 ± 0.10	0.47 ± 0.17								
na^{P890}	0.13 ± 0.11	0.26 ± 0.10	0.31 ± 0.19									
na^{har85}	0.06 ± 0.02	0.21 ± 0.06										
na^{har38}	0.04 ± 0.03											

RK$_2$, *CO*$_2$ and *CO*$_1$ represent deficiencies *Df(1)12C6; 12E5, Df(1)12AB; 12E5-E6* and *Df(1)12D2-E1; 13A2-E5*, respectively. The table represents the average response (±SD) of a minimum of 10 runs and in some cases up to a maximum of 50 runs. In the case of excisions and their various combinations, where the background was ry^{506}, the anesthetic was stopped after 20 min and response was calculated as the ratio of flies eluted in first 20 min to the total number of flies. In all other cases which are ry^{+}/ry^{+} or ry^{+}/ry^{-} the response indicated is the ratio of flies eluted in first 30 min to the total number of flies.

TABLE 4

Geotactic response of flies bearing different alleles and their various combinations

	na^{har38}	na^{har85}	na^{P890}	na	na^{Pex1}	na^{Pex2}	CO_1	CO_2	RK_2	CS	na^{Pex3}	na^{Pex4}
na^{Pex4}	0.88 ± 0.03	0.88 ± 0.01	0.88 ± 0.02	0.89 ± 0.01	0.89 ± 0.01	0.89 ± 0.02	0.90 ± 0.01	0.85 ± 0.01	0.84 ± 0.01	0.86 ± 0.01	0.88 ± 0.01	0.87 ± 0.01
na^{Pex3}	0.89 ± 0.04	0.88 ± 0.02	0.89 ± 0.01	0.89 ± 0.01	0.90 ± 0.02	0.90 ± 0.02	0.89 ± 0.05	0.85 ± 0.01	0.85 ± 0.01	0.87 ± 0.01	0.89 ± 0.02	
CS	0.87 ± 0.01	0.87 ± 0.01	0.87 ± 0.01	0.86 ± 0.01	0.88 ± 0.01	0.87 ± 0.01	0.87 ± 0.01	0.85 ± 0.02	0.89 ± 0.02	0.89 ± 0.01		
RK_2	0.01 ± 0.01	0.02 ± 0.01	0.03 ± 0.01	0.13 ± 0.04	0.14 ± 0.05	0.27 ± 0.04						
CO_2	0.01 ± 0.01	0.02 ± 0.01	0.03 ± 0.01	0.10 ± 0.04	0.25 ± 0.05	0.26 ± 0.05						
CO_1	0.0 ± 0.0	0.01 ± 0.01	0.03 ± 0.01	0.11 ± 0.04	0.16 ± 0.05	0.22 ± 0.05						
na^{Pex2}	0.14 ± 0.03	0.13 ± 0.03	0.11 ± 0.03	0.20 ± 0.04	0.24 ± 0.05	0.25 ± 0.06						
na^{Pex1}	0.08 ± 0.02	0.11 ± 0.02	0.15 ± 0.02	0.15 ± 0.03	0.21 ± 0.05							
na	0.04 ± 0.01	0.08 ± 0.01	0.16 ± 0.02	0.13 ± 0.05								
na^{P890}	0.02 ± 0.01	0.03 ± 0.01	0.04 ± 0.01									
na^{har85}	0.01 ± 0.01	0.01 ± 0.01										
na^{har38}	0.0 ± 0.0											

The table represents the average response (±SD) of 10 runs. The response is calculated as in Table 1.

of mutant tissue in all the individual mosaic flies and their behavior in the inebriometer and geotaxis apparatus was as shown in Figures 4 and 5. In this analysis, only those flies that remained in the column after 30 min, which would translate to a response index of 0.0, were considered resistant. Similarly for geotaxis we considered phenotypically mutant only those flies that remained in the first tube. We also analyzed the behavior of the mosaics in another manner. We pooled together flies that had all of the head of mutant origin to assess the behavior of this lot as a group. This group gave an index nearly the same as that obtained for the mutant fly. Grouping flies that have only the right side of the head mutant or just the left side mutant showed that such groups behaved like wild type. Similarly flies that had all of the body mutant except the head, when seen as a group, behaved as wild type. The straightforward conclusion from the behavior of these mosaics is that the anesthetic resistance has a focus in the head. The blastoderm fate mapping (HOTTA and BENZER 1970, 1972; HALL 1979; ARNOLD and KANKEL 1981) of the mosaics, based on the percent probability of the association of halothane resistance phenotype with certain markers, indicated that the focus in question was very near the ocellar bristle (OC), palp (PA) and antenna (AN) for na^{har38}. The distances for geotaxis behavior caused by na^{har38} and both the behaviors for na^{har85} were also close to those seen for halothane resistance (Table 5). Plotting this hypothetical point with respect to these three markers showed that the focus of halothane-resistance behavior was in the blastoderm region corresponding to the cephalic ganglion and was far from the thoracic ganglion. The distance between the left and right focus is variable and on the average 29.5 sturts, indicating that the focus is not a single point in the cephalic ganglion. The sturt values for the two alleles and the two behaviors showed a scatter owing to the small number of mosaics. However they were close enough and it is likely that the aberrant walking behavior also maps to this focus. An analysis of the region responsible for the behavior of na^{har85} with a few mosaics yielded similar results.

DISCUSSION

New alleles and new phenotypes of *na*: We report here characterization of several mutant alleles of a halothane response locus. The complementation and deficiency mapping data presented in Tables 3 and 4 unambiguously show that na^{har38}, na^{har85}, *na*, na^{P890}, na^{Pex1} and na^{Pex2} are alleles of the same locus. The phenotypes caused by all alleles are recessive, and the behavior of transheterozygotes indicates that they are likely to be loss-of-function or null alleles. This is underscored by the different independent origins of the six alleles: na^{har38} and na^{har85} are EMS induced, *na* is x-ray-induced, na^{P890} is an insertional allele, and na^{Pex1} and na^{Pex2} are

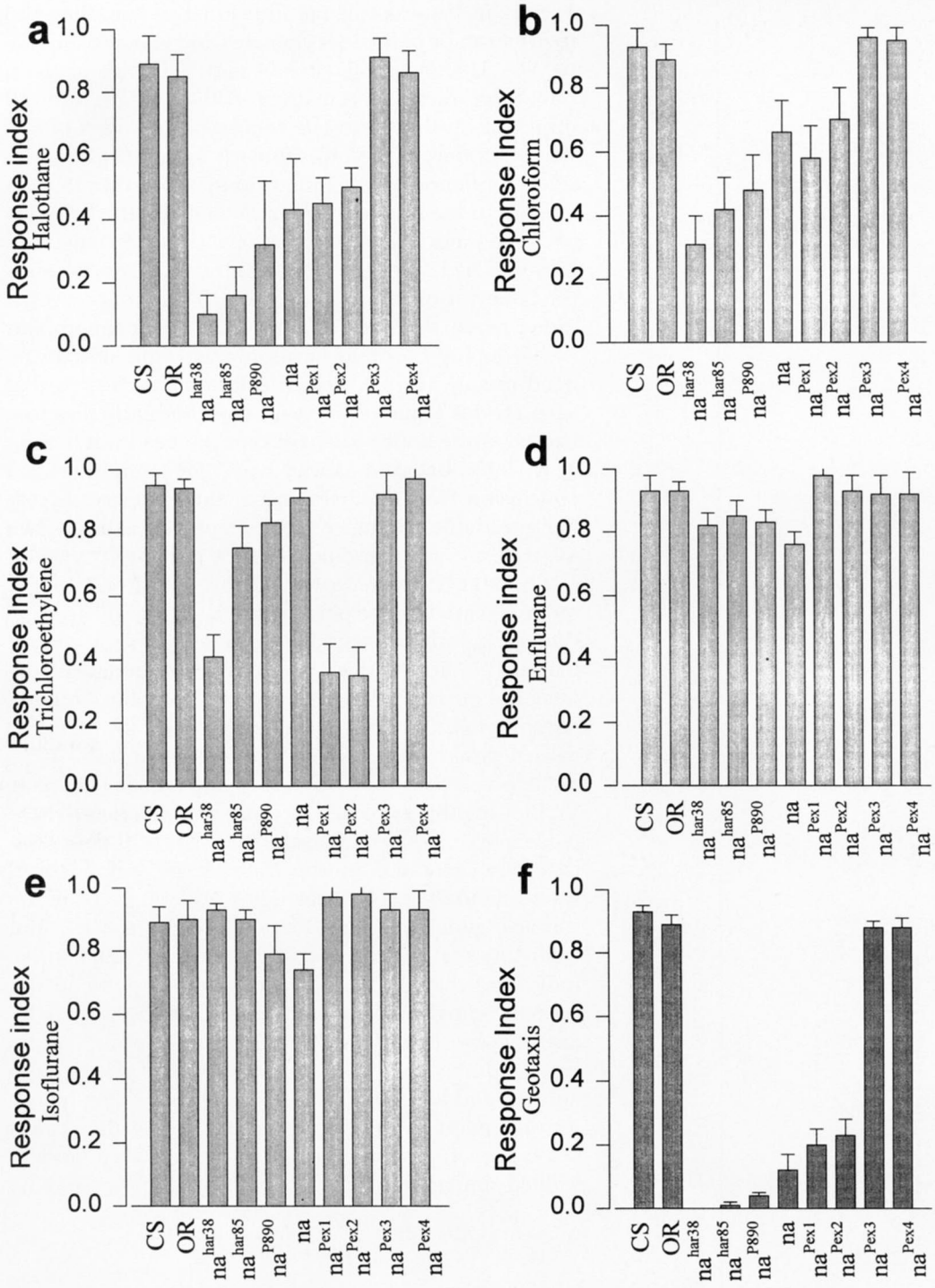

FIGURE 3.—Response of *na* mutants to various inhalational anesthetics (a–e). Flies were tested for their responses to different anesthetics at a concentration of 0.5% as measured by Riken gas meter and response index calculated as in Table 1. Bars represent the average response (±SD) of a minimum of eight runs. Geotaxis deficit in *na* alleles is illustrated in f for comparison. The results (a–f) are for male flies hemizygous for the *na*. The results of behavioral assays with female flies are very similar.

excision alleles. All the alleles caused halothane anesthesia resistance, the saccadic walking behavior and for some alleles, a pronounced narrow abdomen. The narrow abdomen and the walking behavior were displayed by flies even when not exposed to the anesthetic. These independent phenotypes confront us with three possibilities regarding the origin of the halothane resistant phenotype. (1) Resistance to anesthesia may arise owing to a limited absorption of the gaseous agent because of geometric considerations such as narrow tracheal passages. (2) The motor behavior might in some way allow the flies to hold on to the baffles while the anesthetic was passed through the inebriometer. (3) Resistance to anesthesia could be caused by *na* altering the actual target for halothane. These are considered in the light of the mosaic data and behavior in different anesthetics we discuss below.

Distinct pathways for onset of anesthesia: The different mutants whether obtained as a result of mutagenesis by X-ray (*na*), *P* insertion (na^{P890}), EMS (na^{har38} and

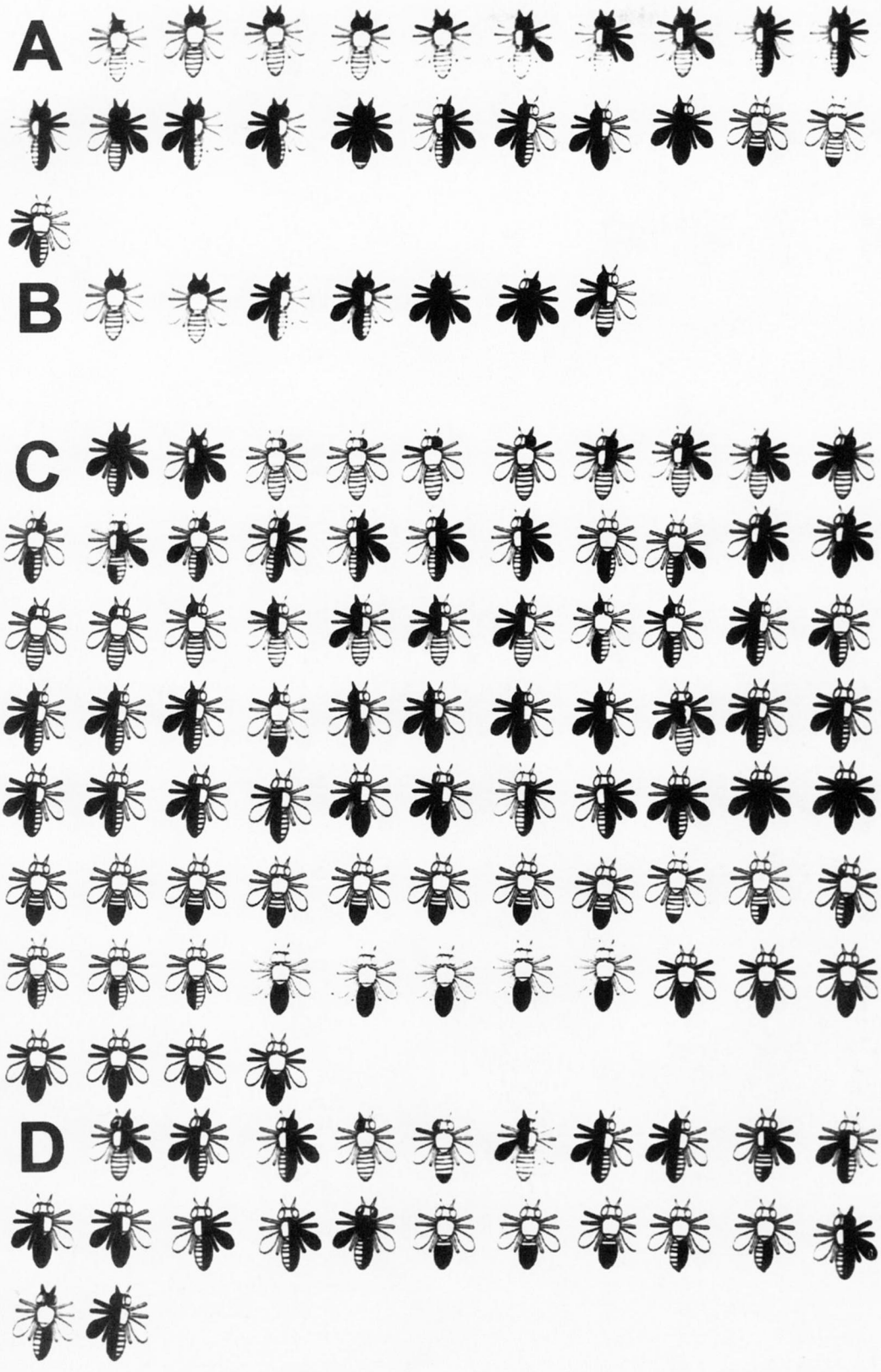

FIGURE 4.—The pattern of mosaics classed by their behavior in the inebriometer. A total of 132 mosaics were obtained after scoring 20,000 potential flies. The mosaics were grouped into classes by behavior (A and B showed mutant behavior). Flies mosaic for the two different alleles, na^{har38} (A and C) and na^{har85} (B and D), are shown separately. The shaded portions indicate mutant tissue as known by cuticular markers, yellow body color and white apricot eye color.

na^{har85}), or as a result of imprecise excisions (na^{Pex1} and na^{Pex2}) behaved similarly. All are resistant to halothane, chloroform and some to trichloroethylene. The differences among alleles in their assorted response to trichloroethylene might reflect a partial loss of function for these alleles compared to the null phenotype of the stronger allele. None of the alleles, however, resulted in measurable resistance to enflurane and isoflurane. The lack of resistance to enflurane- and isoflurane-induced anesthesia in the case of all the *na* mutants indi-

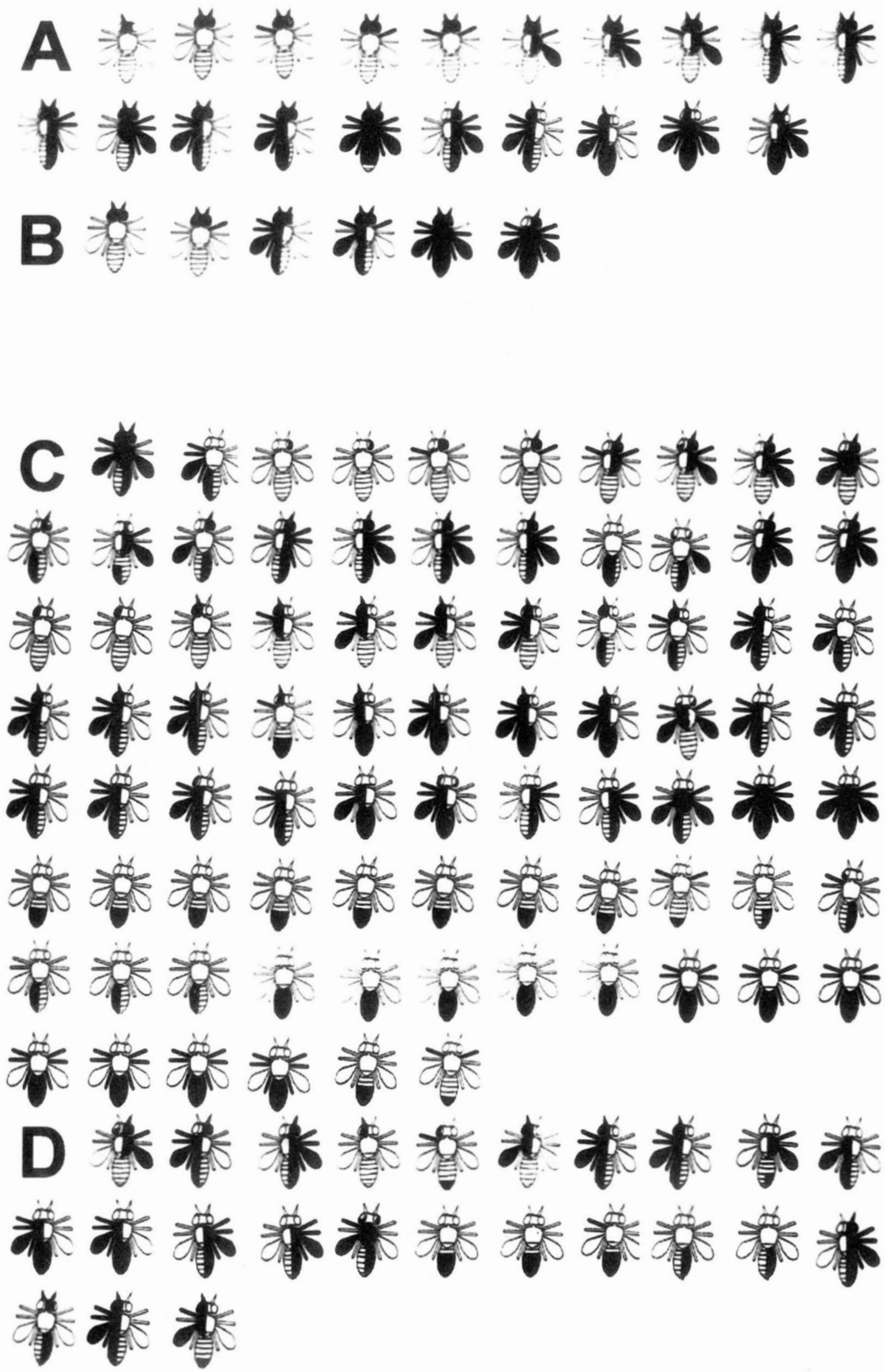

FIGURE 5. — Mosaics classed by their behavior in the geotaxis apparatus. The details are as in Figure 4.

cates clearly that the narrow abdomen does not restrict the availability of inhalational anesthetics to the fly. This result argues that resistance to halothane is not due to geometric considerations that should equally affect all volatile anesthetics. The fact that all the *na* mutants and mosaics that showed resistance to halothane-induced anesthesia also exhibited the walking deficit but remained sensitive to enflurane and isoflurane indicates that the altered motor behavior does not cause a general nonspecific behavioral change that allows flies to

TABLE 5

Blastoderm fate mapping of Horka mosaics

Distance to behavioral focus from	na^{har38} halothane response (sturts)	na^{har38} geotaxis response (sturts)	na^{har85} halothane response (sturts)	na^{har85} geotaxis response (sturts)
Ocellar bristle	7.6	5.8	6.6	3.3
Palp	3.7	6.1	9.9	3.3
Antennae	7.5	5.8	6.6	3.3
Distance between right and left foci	29.5	27.9	23.2	29.9

Different types of mosaics, generated by Horka, were tested for both anesthetic resistance and geotactic response along with control cohorts emerging from the following cross: *y* w^a na^{har38}/*y* w^a na^{har38}; +/+; +/+ × +/*Y*; +/+; *mwh Horka e/TM3* y^+ $ri^{(1)}$ *P(p) sep Sb bx e Ser.* Each time few mosaics of different types were tested along with large number of control cohorts. Those of the mosaic flies that stayed back in the inebriometer after a 30-min elution with the anesthetic were considered resistant. Similarly those of the mosaics that stayed back in the first tube in the counter current apparatus alone were counted as mutant.

be retained in the inebriometer column. The *na* gene product, however, is in some ways involved in motor control. Such a neural deficit may cause the adult musculature to behave in an unusual manner so as to give a narrow abdominal appearance. We do not wish to speculate on the abdominal phenotype since it has not been quantified and is very difficult to follow in all *na* mutants. The two revertants obtained by excision reverted the phenotypes with respect to all of the anesthetics and the geotaxis response. Together, this indicates that the product of *na* is a crucial element in the pathway of onset of anesthesia caused by halothane, chloroform and trichloroethylene. This element has no role in anesthesia induced by enflurane and isoflurane. It is to be noted that the different degrees of response associated with the various alleles are restricted to a set of anesthetics. Here the discrimination appears to be on the basis of the chemical nature of anesthetics because enflurane (CHF_2OCF_2CHClF) and isoflurane ($CF_3CHClOCHF_2$) are chemically isomeric ethers and different from the other anesthetics studied in this report. It is worth mentioning that the spectrum of anesthetics that na^{har38} is resistant to is quite similar to the spectrum of anesthetics to which a mutant in the worm *C. elegans* is hypersensitive. This suggests to us two distinct pathways for the action of these two groups of anesthetics. This result invalidates any identity of mechanisms of action of different anesthetics that may be implied by the unitary target hypothesis.

A focus for response to halothane: While it has been accepted that general anesthetics render animals reversibly unconscious by acting at the central nervous system, the issue of whether there is any specific region or focus for anesthetic action is contentious. Such a region, an aesthesis center, responds to inhalational general anesthetics by producing a state of unconsciousness. Resistance to anesthetic agents could arise due to the immunity of this center to anesthetic action. Mosaic analysis suggested itself as a unique approach to investigating the existence of such a center. We generated chimeric flies that have parts of their nervous system or other body parts that are genotypically mutant in an otherwise wild-type background. We then investigated the behavior of such flies in detail. These would provide us answers to two types of questions: Whether resistance to anesthetics derives from mutant abdomen or thorax possessing specific motor disabilities ("sticky leg") or specific degradative abilities to destroy classes of anesthetics ("detoxification"). In light of the distinct responses to different anesthetics the first alternative is unlikely. Similarly it is difficult to imagine a single degradative component that will render flies resistant to halothane and chloroform while leaving its response to enflurane and isoflurane intact. However, mosaic animals allowed us to directly examine the contribution of different tissues to anesthesia resistance in na^{har38} and na^{har85} mutants. The results of this analysis were reasonably conclusive. Evidently flies that have all of their head or most of it derived from the mutant tissue showed resistance to halothane and abnormal walking behavior indistinguishable from the mutant. As a group flies with large or small patches of mutant tissue covering the rest of the body, including the thoracic ganglion and either the left or right side of the head, behaved like wild type. From a blastoderm fate mapping of the data, the altered response in *na* mutants seems to derive from a center in the cephalic ganglion. Although we cannot say much about how small a region this is, obviously it is exclusively in the cephalic ganglion. We believe this is also the primary center for general anesthetic action. This is a rather unique observation for general anesthetics. All the studies have implicated the central nervous system but this is the only clear demonstration of a center in the brain for a response related to general anesthesia. The mosaic analysis in flies implicates specifically a small part of the central nervous system in the altered response to the anesthetics and eliminates the role of effector organs.

Our conclusion that anesthetics act preferentially on a subset of neuronal cells is consistent with recent elec-

trophysiological studies. LIN and NASH (1996) reported that anesthetics preferentially abolish long latency responses obtained from muscles when the fly CNS is subjected to a suprathreshold electrical stimulation. Studies using decapitated flies showed that removal of the cephalic ganglion altered their response to volatile anesthetics. Mutants that were hypersensitive were reverted to relatively normal behavior by removal of the head (LEIBOVITCH *et al.* 1995). Removal of heads rendered wild-type flies resistant to volatile anesthetics when compared to their more fortunate siblings. These data are generally consistent with the notion that the mutant effects on the cephalic ganglion are what cause their aberrant responses to halothane anesthesia. We must, however, point out that while *har56* lost its resistance upon decapitation na^{har38} did not do so. This indicates that while anesthetics may act preferentially on subsets of neurons, they probably affect, to a lesser or greater extent, all cells in the nervous system. It is also possible that more than one center for aesthesis exists in the brain and elsewhere and they act additively in the intact fly.

A second interesting feature of our mosaic analysis is that the aberrant walking behavior (motor defect) of *na* mutants also mapped to the same region as the resistance to anesthetics. This result implies that the coordination of walking behavior that occurs in the thoracic ganglion is under control of the cephalic ganglion. The rather unusual, saccadic walking pattern shown by *na* mutants suggests that there may be a variety of subtle controls of motor behavior exercised via cephalic input. Whether the cells that exercise these controls are the same as those most responsive to volatile anesthetics is an open question, but this hypothesis is consistent with our fate mapping data, performed with full knowledge of its limitations for spatial resolution within the cephalic ganglion. However, it is interesting to note that *unc-79* and *unc-80* mutants in *C. elegans* also show a defective locomotion, described as "fainters," in the absence of anesthetics (MORGAN *et al.* 1988). It is possible that the focus for resistance mapped by us is a neural center, which is hierarchically at a higher level in the motor control system and the most sensitive element in the pathway of anesthesia. Further analysis with a variety of mutants will be rewarding.

We owe much to HOWARD NASH for his inspiration. We gratefully acknowledge his advice at various stages of this effort. We thank VERONICA RODRIGUES for useful discussions. Thanks are due to the two anonymous referees for their very useful comments and constructive criticism that helped improve the manuscript considerably. This work was supported by Department of Science and Technology grant DST/SP/SO/N11-91 to K.S.K. and M.R. and Human Frontier Science Programme grant to M.R., K. VIJAYRAGHAVAN and MIKE BATE. M.R. is an Alfred. P. Sloan Research Fellow and a McKnight Neuroscience Scholar.

LITERATURE CITED

ANAND, A., J. FERNANDES, M. C. ARUNAN, S. BHOSEKAR, A. CHOPRA *et al.*, 1990 Drosophila "enhancer-trap" transposant gene expression in chemosensory and motor pathways and identification of mutants affected in smell and taste ability. J. Genet. **69:** 151–168.

ARNOLD, J., and D. R. KANKEL, 1981 Fate mapping multifocus phenotypes. Genetics **99:** 211–229.

BENZER, S., 1967 Behavioral mutants of Drosophila isolated by counter current distribution. Proc. Natl. Acad. Sci. USA **58:** 1112–1119.

CAMPBELL, D. B., and H. A. NASH, 1994 Use of Drosophila mutants to distinguish among volatile general anesthetics. Proc. Natl. Acad. Sci. USA **91:** 2135–2139.

GILMAN, A. G., T. W. RAIL, A. S. NIES and P. TAYLOR, 1991 *Pharmacological Basis of Therapeutics.* Pergamon Press, New York.

HALL, J. C., 1979 Control of male reproductive behavior by central nervous system of Drosophila: dissection of a courtship pathway by genetic mosaics. Genetics **92:** 437–457.

HOTTA, Y., and S. BENZER, 1970 Genetic dissection of the Drosophila nervous system by means of mosaics. Proc. Natl. Acad. Sci. USA **67:** 1156–1163.

HOTTA, Y., and S. BENZER, 1972 Mapping behavior in Drosophila mosaics. Nature **240:** 527–536.

KRISHNAN, K. S., and H. A. NASH, 1990 A genetic study of the anesthetic response: mutants of *Drosophila melanogaster* altered in sensitivity to halothane. Proc. Natl. Acad. Sci. USA **87:** 8632–8636.

LEIBOVITCH, B. A., D. B. CAMPBELL, K. S. KRISHNAN and H. A. NASH, 1995 Mutations that affect ion channels change the sensitivity of *Drosophila melanogaster* to volatile anesthetics. J. Neurogenet. **10:** 1–13.

LIN, M., and H. A. NASH, 1996 Influence of general anesthetics on a specific neural pathway in *Drosophila melanogaster.* Proc. Natl. Acad. Sci. USA **93:** 10446–10451.

MEYER, H. H., 1899 Theorie der Alkaholnarkose I Mitt. Welche Eigenschaft der Anasthetika bedingt ihre Narkotische Wirkung Arch. Exper. Pathol. Pharmak. **42:** 109–119.

MILLER, H. M., 1934 Dros. Info. Serv. **2:** 9.

MIR, B. A., and K. S. KRISHNAN, 1995 Genetic approaches to study of anesthesia. Curr. Sci. **68:** 1214–1221.

MORGAN, P. G., M. M. SEDENSKY, P. M. MENEELY and H. F. CASCORBI, 1988 The effect of two genes on the anesthetic response in the nematode *Caenorhabditis elegans.* Anesthesiology **69:** 246–251.

MORGAN, P. G., and M. M. SEDENSKY, 1994 Mutations conferring new patterns of sensitivity to volatile anesthetics in *Caenorhabditis elegans.* Anesthesiology **81:** 889–898.

NASH, H. A., D. B. CAMPBELL and K. S. KRISHNAN, 1991 New mutants of Drosophila that are resistant to the anesthetic effects of halothane. Ann. NY Acad. Sci. **625:** 540–544.

OVERTON, E., 1901 Studien uber die Narkose. Jena Verlag von Gustav Fischer, Frankfurt, Germany.

PALLANCK, L., R. W. ORDWAY, M. RAMASWAMI, W. Y. CHI, K. S. KRISHNAN *et al.*, 1995 Distinct roles for N-Ethylmaleimide-sensitive fusion protein (NSF) suggested by the identification of a second Drosophila NSF homolog. J. Biol. Chem. **270:** 18742–18744.

SEDENSKY, M. M., and P. M. MENEELY, 1987 Genetic analysis of Hlothane sensitivity in *Caenorhabditis elegans.* Science **236:** 952–954.

SZABAD, J., E. MATHE and J. PURO, 1995 *Horka,* a dominant mutation of Drosophila induces non disjunction and through paternal effect, chromosome loss and genetic mosaics. Genetics **139:** 1585–1599.

WEBER, K. E., 1988 An apparatus for measurement of resistance to gas-phase reagents. Dros. Info. Serv. **67:** 91–93.

Communicating editor: V. G. FINNERTY