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Common Genetic Determinants of Halothane and Isoflurane Potencies in Caenorhabditis elegans

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Background: **Genetics provides a way to evaluate anesthetic action simultaneously at the molecular and behavioral levels. Results from strains that differ in anesthetic sensitivity have been mixed in their support of unitary theories of anesthesia. Here the authors use the previously demonstrated large variation of halothane sensitivities in *Caenorhabditis elegans* recombinant inbred strains to assess the similarities of the determinants of halothane action with those of another volatile anesthetic, isoflurane.**

Methods: **The recombinant inbred strains, constructed from two evolutionarily distinct *C. elegans* lineages, were phenotyped. A coordination assay on agar quantified the sensitivity to the volatile anesthetics; median effective concentrations (EC_{50}s) were calculated by nonlinear regression of concentration–response data and were correlated between the drugs for those strains tested in common. Genetic loci were identified by statistical association between EC_{50}s and chromosomal markers.**

Results: **The recombinant inbred strains varied dramatically in sensitivity to halothane and isoflurane, with a 10-fold range in EC_{50}s. Heritability estimates for each drug were imprecise but altogether high (49–80%). Halothane and isoflurane EC_{50}s were significantly correlated ($r = 0.71$, $P < 10^{-9}$). Genetic loci controlling sensitivity were found for both volatile anesthetics; the most significant determinant colocalized on chromosome V. A smaller recombinant inbred strain study of ethanol-induced immobility segregated different genetic effects that did not correlate with sensitivity to either halothane or isoflurane.**

Conclusions: **The genetic determinants driving the large variation in anesthetic sensitivity in these *C. elegans* recombinant inbred strains are very similar for halothane and isoflurane sensitivity. (Key words: Recombinant inbred strains; volatile anesthetics; ethanol; nematodes; theories of anesthesia.)**

UNITARY theories of anesthesia predict that all volatile anesthetics (VAs) depress the nervous system by the same mechanism. Genetics provides a powerful, and at present the only, tool to test this hypothesis. Although limited, genetic studies in nematodes, flies, and mice have not supported a unitary theory of anesthesia. In the nematode *Caenorhabditis elegans* using an anesthetic end point requiring supraclinical VA concentrations, two mutant strains were isolated that were hypersensitive to halothane but normally sensitive to isoflurane and other VAs.[1–3] Subsequently, additional mutations were found that produced divergent effects on various VA potencies, but other mutations were isolated that conferred hypersensitivity to all VAs tested.[4] Thus, these data from *C. elegans* are mixed, suggesting that some components of the anesthetic mechanism operating at these concentrations are shared by all VAs, and some are unique to each drug.

In *Drosophila melanogaster*, halothane-resistant mutant strains with an abnormal sensitivity to clinically relevant concentrations of halothane have been isolated.[5,6] The potencies of anesthetics of different chemical structure, notably halothane *versus* isoflurane, were found to be affected to different extents by one or more halothane-resistant mutations.[6] However, all departures from wild-type VA sensitivity were relatively small (less than two times).

Some natural variation in VA sensitivity in rodents has been found.[7–10] Mouse lines selected for short sleep and long sleep after hypnotic doses of ethanol also were found to differ slightly but significantly in sensitivity to isoflurane and enflurane[11] but not to halothane.[12] Rat lines with different sensitivities to ethanol were also differentially sensitive to halothane, isoflurane, and enflurane.[10] However, the VA median effective concentration (EC_{50}) differences were only approximately 20–40%. These small differences allow for the possibility

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that the major determinants of VA action are not divergent in these lines.

We showed previously that *C. elegans* recombinant inbred strains (RIs) express large differences in halothane sensitivity as a result of the effect of several genes.[13] These strains are not mutants but are composed of distinct genetic mixtures of two wild-type strains, N2 and BO. The amount of N2 *versus* BO DNA sequence divergence appears to be small, except for the many transposon insertions in the BO strain.[14,15] Nevertheless, the RI strains express a more than 10-fold range of halothane sensitivities for loss of coordinated movement,[13] an anesthetic end point that occurs rapidly and reversibly at "clinical" (*i.e.*, concentrations used for human anesthesia) VA concentrations (*C. elegans* halothane EC_{50} = 0.3 to 1.1 vol% at 20°C, depending on assay conditions and wild-type strain; see materials and methods).[16,17]

Here we ask whether the genetic determinants that control the large differences in halothane sensitivity in these RI strains similarly control isoflurane sensitivity. This question is addressed at two levels: first, whether the halothane and isoflurane EC_{50}s correlate significantly in the genetically distinct RI strains, and second, whether the genetic loci responsible for the differences in VA potency colocalize for the two drugs. Using an immobility assay different from the assay used to measure VA-induced loss of coordination, we also determined the sensitivities to ethanol of a subset of the RIs to assess mechanistic similarities with VAs.

Materials and Methods

Nematode Strains

We used standard methods to maintain *C. elegans* strains as described by Brenner.[18] All strains were grown at 20°C on standard nutrient growth media plates with *Escherichia coli* strain OP50 as a food source.[18] Construction of RI lines derived from two isogenic wild-type *C. elegans* strains, Bristol-N2 and Bergerac-BO, was described previously.[19–21] Because the RI strains are homozygous, they are relatively genetically stable and, consequently, can be tested repeatedly for various phenotypic end points. However, the BO strain has a small incidence of spontaneous mutation caused by transposition[15] that may increase the variance between replicate phenotypes of BO and the RIs. Standard *C. elegans* nomenclature is used throughout.[22]

Behavioral Assays

Behavioral experiments were performed only on well-fed young adult worms that had not been through a dauer larva phase.[18] Two kinds of behavioral assays were performed to determine anesthetic sensitivity phenotypes of the RI strains and the parental lines. The behavioral end point comparing halothane and isoflurane sensitivity was loss of coordinated movement as measured by a radial dispersal assay that was performed identically in this and the previous halothane quantitative trait locus (QTL) study.[13] The worms were washed off the nutrient growth media plates with 1 ml S-basal into Eppendorf tubes and subsequently washed twice with 1 ml S-basal and once with water. After the washes, the 400–600 worms were resuspended in 100 μl water, where they remained (never for > 30 min) until assayed. Ten-microliter aliquots of worms (approximately 50–100) in water were spotted onto the center of 9.5-cm nutrient growth media agar plates with a ring of OP50 *E. coli* at the edges. The assay plates were placed into glass chambers, and various amounts of liquid VA were injected and allowed to vaporize into the air-tight chambers. After dissipation of the water on the assay plate (typically 5 min), the plate was briefly shaken for approximately 5 s to separate the worms from a single clump, and the animals were allowed to disperse to a bacterial ring for 45 min. A ratio of worms in the ring to total number of worms defined the dispersal index. In the absence of anesthetic, approximately 90% of the wild-type worms reached the ring of bacteria approximately 4 cm away. The range of dispersal indices in the absence of anesthetic in the RIs was 45–100%. Gas-phase anesthetic concentrations were determined at the end of the assay by gas chromatography by interpolating between known standards and were always within the linear range of the flame ionization detector and integrator. Halothane and isoflurane both cause a concentration-dependent marked uncoordination of the worms that prevents them from getting to the ring.[16] Shaking the plates at the beginning of the assay, a modification of a previously published protocol,[16] induces the animals to disentangle from a clump that may interfere with the uncoordination phenotype intending to be assayed. This measure of VA-induced uncoordination produces a higher halothane EC_{50} (0.45 ± 0.02) than previously was reported for the radial dispersal assay executed without shaking (0.32 ± 0.05 vol%).[16] An additional modification from the original protocol was instituted to stagger the times for scoring the plates. The worms from a single tube were spotted in 5-min intervals, so they sat for variable periods in the

water. Subsequently we discovered that incubation in water increases the halothane EC_{50} to 0.75 vol%.[17] Shaking the plates and incubation in water account for the increase of the EC_{50} from that previously reported.[16,17]

Ethanol sensitivity was scored as described by Eckenhoff and Yang.[23] Worms were washed from the plate, as in the dispersal assay, except that the final two washes were in ethanol at the appropriate concentration (0, 300, 600, or 900 mM). The worms were incubated for 20 min in the ethanol solution, and then approximately 100 worms were placed in pipettes in 1 ml ethanol solution to 1.5-cm microtiter wells, where movement of 40–70 young adult worms was scored after a 5-min wait. A movement score for the group was obtained by observing each worm for 10 s and assigning it a numeric score: not moving (0), slowly moving (1), or rapidly moving (2). The fraction of animals in each category multiplied by the score and summed yields a semiquantitative movement index for the strain from 0 to 2. These measures of ethanol-induced immobility were contrasted to a previously gathered data set for halothane-induced immobility. A total of 32 RIs were tested in common for ethanol and halothane sensitivity by the immobility end point. For the dispersal end point, 57 RIs were tested in common for halothane and isoflurane sensitivity both; thus, these 57 RIs are used for correlation of sensitivity to the two VAs. For QTL mapping, a previously published data set[13] of 73 RIs was used for halothane, 57 strains for isoflurane, and 33 strains for ethanol sensitivity.

Concentration–Response Curves and Statistics

Behavioral experiments were performed at various concentrations of halothane or isoflurane to generate a concentration–response curve for each RI strain. These included at least five concentrations of VA and one air control. Concentration–response data were fit by nonlinear regression (Fig. P; Biosoft, Cambridge, United Kingdom) according to the equation $y = (\min + (\max - \min))/(1 + [x/x_{50}]^{-k})$ where y = the anesthetic end point score, x = [anesthetic], and k = slope. Any curves resulting in a poor standard error of the estimated EC_{50} (SE > 20%) were retested, and all points subsequently were combined for a new fit. Ethanol EC_{50}s were estimated similarly, although each curve consisted of identical four ethanol concentrations (0, 300, 600, 900 mM ethanol) for all RIs. All RI strains were tested blindly with respect to performance in other anesthetic experiments. To assess significant resistance or hypersensitivity compared with the wild-type strain, the concentration–response data from an RI strain were simultaneously fit with that of the wild-type strain N2, as described by Waud[24] and as implemented by Delean *et al.*[25] (using the National Institutes of Health Allfit curve-fitting program), and correlations among RIs for their sensitivities to different anesthetics were determined by the Pearson correlation coefficient (SYSTAT statistical package; SPSS, Chicago, IL). Significance for all tests was set at $P < 0.05$.

Broad-sense heritabilities were calculated by the equation $H^2 = V_g/(V_g + V_e)$, where $V_g = (V_p - V_e)/2$. This ratio provides an estimate of the proportion of RI phenotypic variance (V_p), which is caused by genetic components (V_g) rather than by environmental variability (V_e).[26] For isoflurane sensitivity, the environmental component, V_e, was estimated by the mean of the different measures of environmental variance: the variance within subsequent EC_{50} measurements of the two parental strains, N2 and BO, and the variance within duplicate EC_{50} measurements of 11 RI strains. For halothane sensitivity, no subsequent measurements of RIs were performed; thus, V_e was estimated by the mean of the N2 and BO variances, as previously described.[13]

Quantitative Trait Locus Mapping

A gene or set of tightly linked genes controlling a quantitative or continuously varying trait such as anesthetic sensitivity is a QTL. A QTL is identified by a significant association between a segment of a chromosome and a variation in phenotype, such as anesthesia EC_{50}s.[27] The genome of each RI is a mixture of adjacent chromosomal segments from either the BO or N2 parent. The parental origin of a particular segment is determined by polymerase chain reaction amplification of marker sequences within that segment that differ in N2 and BO.[28] The genotype of 27 DNA markers that differ between N2 and BO and span 55% of the *C. elegans* genome were determined for each RI, as described previously.[13] A significant association or linkage of a marker to variations in anesthetic EC_{50} (*i.e.*, anesthesia QTLs) was sought using a regression algorithm of marker against EC_{50} (QTL CARTOGRAPHER mapping program; North Carolina State University, Raleigh, NC).[29,30] Table 1 lists the F ratios and the probability values of the regression, assuming a normal distribution of the data. However, QTL significance was determined empirically by experiment-wise permutation of phenotypic data against the genotypes, as previously described.[13,31] Significance was set at an α level less than 0.05. This conservative method of determining significance levels does not assume that the data are distributed normally.[31]

Table 1. QTLs for Dispersal Anesthesia

Nearest Marker*	Anesthetic	Mean EC_{50} RIs (vol%)† BO Allele	Mean EC_{50} RIs (vol%)† N2 Allele	F Ratio‡	*P* Value
stP124 (IC)	Halothane	1.12 ± 0.12	0.69 ± 0.05	16.8	0.0001
stP6 (VR)	Halothane	0.58 ± 0.05	1.01 ± 0.07	23.2	0.000008
stP6 (VR)	Isoflurane	0.62 ± 0.07	0.94 ± 0.05	13.1	0.0006

Interacting Markers§		Genotype *stP23*	Genotype *stP2*	Mean EC_{50} [RIs (mM)]	F Ratio	*P* Value
stP23̄ (VC) × *stP2* (XR)	Ethanol	N2	N2	453 ± 19	15.0	0.0006
		N2	BO	458 ± 31		
		BO	N2	385 ± 31		
		BO	BO	616 ± 34		

QTL = quantitative trait locus.

* Chromosomes I, II, III, IV, V, X; left (L), center (C), right (R).

† Mean ± SEM of the EC_{50} for all strains with either the BO or the N2 allele at that marker.

‡ Single QTL F ratios and *P* values are by marker regression (QTL CARTOGRAPHER Mapping Program, N.C. State Univ.). Raw *P* values are given; only single QTLs significant at $P < 0.05$ by permutation[31] are shown.

§ Interacting loci are those where the phenotypic effect due to variation at one locus is markedly enhanced or repressed by another distinct locus (*i.e.*, the marker effects are not merely additive). The F ratio and *P* value are for the interaction between the loci. Bonferroni-corrected significance thresholds are 0.05/55 = 0.0009.

Genetic interactions between two QTLs were sought by analysis of variance (SYSTAT; SPSS) using the equation EC_{50} = constant + marker1 + marker2 + (marker1 × marker2).[13] Because of the lack of permutation algorithms for interacting QTLs, the threshold for significance of the interaction term was Bonferroni corrected and set at an α level less than 0.0009 ($\alpha < 0.05$ of 55 possible pairwise tests of 11 independent linkage clusters).[20] Significant QTLs were further mapped by an interval-mapping algorithm that interpolates the genetic effect between markers (Zmapqtl, model 3, simple interval mapping QTL CARTOGRAPHER program).[29,30] The interval mapping method provides a more precise localization of QTL.[29]

Results

Phenotypic Variance and Heritability

Figure 1 shows the distributions of sensitivities among the RI strains (strain distribution patterns [SDPs]) for halothane and isoflurane. The RI strains showed a large phenotypic variance for both anesthetic agents. The variance in EC_{50}s for both anesthetics was much greater in the RIs ($P < 0.01$) than in the isogenic wild-type parents (the square root of the variance, or standard deviation, is shown in figure 1). The larger RI variance indicates that genetic, rather than environmental, determinants must be controlling much of the RI V_p because isogenic variance represents that resulting from the environment. The fraction of phenotypic variance caused by genetic determinants is estimated by broad-sense heritability (H^2).[26] The heritabilities were substantial for both drug effects: 78% for halothane and 49% for isoflurane. Because the isoflurane calculations included a more-thorough assessment of within-strain repeatability, its heritability estimate is probably more accurate than the halothane estimate. Repeated measurements did show a large environmental component, as can be visualized by the scattered EC_{50}s derived for the N2 parental strain in halothane and isoflurane (fig. 1). After these experiments were completed, we found that the time the worms sat in water before being placed on the agar assay plates greatly contributed to the environmental variance (see materials and methods)[17]; this uncontrolled portion of the environmental variance is likely to have similarly influenced the RI phenotypes. The large parental variance notwithstanding, significantly resistant and hypersensitive RIs were identified for both VAs (table 2). Retesting of some of the RIs controlling for the temporal effect by immediate spotting found that sensitive strains were still sensitive and resistant strains remained (albeit less) resistant (data not shown).

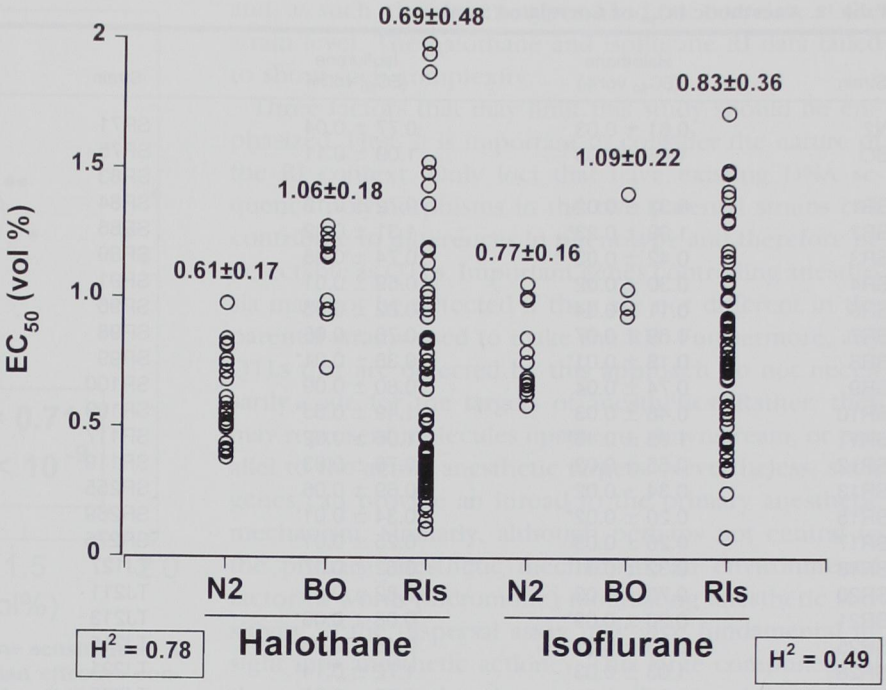

Fig. 1. Distribution of anesthetic sensitivities in N2 and BO wild-type strains and the recombinant inbred strains (RIs). Distribution for the 57 RIs tested in common for halothane and isoflurane anesthesia. Each circle represents a different median effective concentration (EC_{50}; vol %) calculated from a concentration–response curve of at least six points. The mean ± SD for each group is given. Broad-sense heritability (H^2) for each anesthetic is calculated as a ratio of genetic *versus* phenotypic variance.[27] The genetic variance is the phenotypic variance (the variance of the RIs) minus the environmental variance. The environmental variance used for the the halothane data set was an average of both parental variances, whereas the isoflurane environmental variance in addition included a within-RI strain variance generated from duplicate EC_{50} measurements of 11 RIs.

Interanesthetic Correlations

Fifty-seven RIs were studied for halothane and isoflurane sensitivity (table 2). We tried to determine whether the same genetic determinants might control sensitivity in the RIs to both VAs by correlating the EC_{50} phenotypes for both drugs. The SDPs for the general anesthetics halothane and isoflurane were significantly correlated ($r = 0.71$, $P < 10^{-9}$), as shown in table 2 and figure 2. Generally, for example, RI strains resistant to halothane were also resistant to isoflurane (SR28, SR66, SR68, SR70, SR83, SR84, SR99, SR255, and TJ221). Duplicate EC_{50}s were determined for 11 strains in the isoflurane data set (data not shown; the EC_{50}s reported for these strains in table 2 are derived from combined data points). This measure of repeatability produced a within-strain correlation statistic ($r = 0.70$) that was remarkably close to the between-anesthetic correlation.

To probe the relation between determinants of VA and ethanol sensitivity, we measured ethanol sensitivity in a subset of these RIs. However, because of the slow volatility of ethanol relative to the 45-min dispersal assay, ethanol sensitivity could not be measured accurately by the dispersal assay, so an immobilization end point in liquid was used. Thus, we primarily compared ethanol immobilization with immobilization by halothane.[1,13,16] The 33 RIs studied for ethanol-induced immobility segregated a large V_p (2.2 times the mean), a large heritability ($H^2 = 0.78$), and hence mapping potential. Ethanol EC_{50} phenotypes did not correlate ($r = 0.23$, $P = 0.21$) with sensitivity to high concentrations of halothane as measured by the immobility assay (fig. 3). They also did not correlate with halothane sensitivity by the dispersal end point ($r = 0.06$, $P = 0.75$). Predictably, the ethanol data did not correlate with the isoflurane data either ($r = 0.19$, $P = 0.31$). However, we must emphasize that these latter two correlations measure drug potency by two completely different assays.

Quantitative Trait Locus Analysis

One QTL mapping onto chromosome V near the *stP6* marker was significant for isoflurane sensitivity in the 57 RIs studied (table 1). We previously reported in a larger halothane study ($n = 73$) that the largest QTL for halothane sensitivity for both the male-mating and the radial dispersal behavioral end points was in the middle of chromosome V near the *stP6* marker.[13] The nonidentical set of 57 RIs used here for correlation with isoflurane confirms that the largest genetic effect for halothane sensitivity is near the *stP6* locus (data not shown). As shown by interval mapping in figure 4, the isoflurane sensitivity QTL peaks at the same location near the *stP6* marker as the halothane sensitivity QTL. The *stP6* locus is genetically distant (24 expanded map units) from a

Table 2. Anesthetic EC_{50} of Correlated Strains

Strain	Halothane (EC_{50} vol%)	Isoflurane (EC_{50} vol%)	Strain	Halothane (EC_{50} vol%)	Isoflurane (EC_{50} vol%)
N2	0.61 ± 0.03	0.77 ± 0.04	SR71	0.65 ± 0.13	0.86 ± 0.09
BO	1.06 ± 0.05	1.09 ± 0.11	SR75	0.93 ± 0.05	1.00 ± 0.02
			SR83	1.14 ± 0.08*	1.40 ± 0.28*
SR1	0.33 ± 0.02	0.72 ± 0.12	SR84	1.53 ± 0.28*	1.15 ± 0.11*
SR2	1.99 ± 0.23*	1.31 ± 0.02	SR86	0.32 ± 0.02	0.41 ± 0.01
SR3	0.42 ± 0.03	0.74 ± 0.08	SR89	0.53 ± 0.01	0.51 ± 0.03
SR4	0.30 ± 0.02	0.59 ± 0.01	SR91	1.01 ± 0.09	1.19 ± 0.16*
SR6	0.11 ± 0.04*	0.82 ± 0.03	SR96	0.35 ± 0.01	0.58 ± 0.07
SR7	0.69 ± 0.07	0.76 ± 0.06	SR98	0.28 ± 0.01	0.56 ± 0.02
SR8	0.18 ± 0.01*	0.38 ± 0.01*	SR99	1.44 ± 0.11*	1.35 ± 0.09*
SR9	0.74 ± 0.04	0.80 ± 0.09	SR100	0.47 ± 0.04	0.83 ± 0.07
SR10	0.46 ± 0.03	1.49 ± 0.05*	SR109	0.41 ± 0.03	0.70 ± 0.02
SR11	1.95 ± 0.46*	1.06 ± 0.02	SR117	0.79 ± 0.06	0.91 ± 0.09
SR12	0.55 ± 0.02	0.78 ± 0.03	SR119	0.84 ± 0.01	1.17 ± 0.06*
SR13	0.34 ± 0.02	0.69 ± 0.06	SR255	1.88 ± 0.15*	1.15 ± 0.11*
SR15	0.20 ± 0.02*	0.34 ± 0.01*	SR269	0.29 ± 0.01	0.59 ± 0.04
SR17	0.26 ± 0.03	0.25 ± 0.01*	SR276	1.03 ± 0.15*	0.75 ± 0.04
SR18	0.32 ± 0.01	0.62 ± 0.07	TJ127	0.76 ± 0.04	1.07 ± 0.03
SR20	0.73 ± 0.03	0.82 ± 0.03	TJ211	0.69 ± 0.08	0.92 ± 0.02
SR21	0.20 ± 0.09*	0.08 ± 0.06*	TJ213	0.25 ± 0.03	0.45 ± 0.02
SR25	0.83 ± 0.01	0.46 ± 0.03	TJ215	0.85 ± 0.01	0.93 ± 0.04
SR28	1.03 ± 0.03*	1.72 ± 0.14*	TJ221	1.49 ± 0.04*	1.35 ± 0.03*
SR29	0.27 ± 0.01	0.88 ± 0.06	TJ223	0.52 ± 0.01	0.58 ± 0.07
SR30	0.36 ± 0.01	1.10 ± 0.1	TJ226	0.70 ± 0.03	0.90 ± 0.10
SR42	0.29 ± 0.01	0.44 ± 0.08	TJ280	0.72 ± 0.11	0.75 ± 0.05
SR54	0.14 ± 0.02*	0.70 ± 0.05	TJ286	0.23 ± 0.01	0.54 ± 0.07
SR58	0.96 ± 0.02	1.01 ± 0.05*	TJ292	0.18 ± 0.03*	0.25 ± 0.01*
SR66	1.19 ± 0.01*	1.50 ± 0.02*	TJ294	0.37 ± 0.02	0.54 ± 0.05
SR68	1.20 ± 0.27*	1.42 ± 0.04	TJ296	0.39 ± 0.02	0.35 ± 0.04
SR70	1.37 ± 0.01*	1.30 ± 0.09*	TJ299	0.40 ± 0.05	0.73 ± 0.05

N2 and BO EC_{50} are mean ± SEM. RI EC_{50} are mean ± standard error of the estimate.

* Significantly different from the N2 wild-type strain at $P < 0.05$.

locus controlling coordination in the absence of anesthetic.[13]

We searched for loci controlling sensitivity to ethanol. Two QTLs were detected, one at the *stP23* marker on chromosome V and the other at *stP2* on the right arm of the X chromosome. The QTLs exerted a significant genetic effect on ethanol sensitivity only by interacting with each other (table 1). Interacting loci are those in which the phenotypic effect caused by variation at one locus is markedly enhanced or repressed by another distinct locus (*i.e.*, the marker effects are not merely additive). Although both on chromosome V, the halothane–isoflurane QTL at *stP6* is distant (9.8 expanded map units) from the ethanol sensitivity locus near *stP23*. Mapping of halothane-induced immobility (as reported previously) failed to identify any significant loci, interacting or otherwise, in *C. elegans* RIs.[13]

Discussion

We showed previously that quantitative genetic analysis of recombinant inbred *C. elegans* strains can be used to map loci controlling sensitivity at a behavioral level to clinical concentrations of halothane.[13] The current study extends our previous work by measuring isoflurane sensitivity in the RIs. We chose to study isoflurane in this context for two reasons. First, we wanted to see whether another VA could reproduce the results that we found for halothane QTL mapping. Second, these RIs with multiple naturally variant genes and a large genetic variance in halothane sensitivity seemed particularly well suited to address the similarity or difference in mechanisms of two different VAs. We also compared the VA EC_{50}s with a smaller ethanol data set to determine whether an entirely different kind of anesthetic shared genetic determinants with either of the VAs. Toward this end, we contrasted the ethanol sensitivity data with two

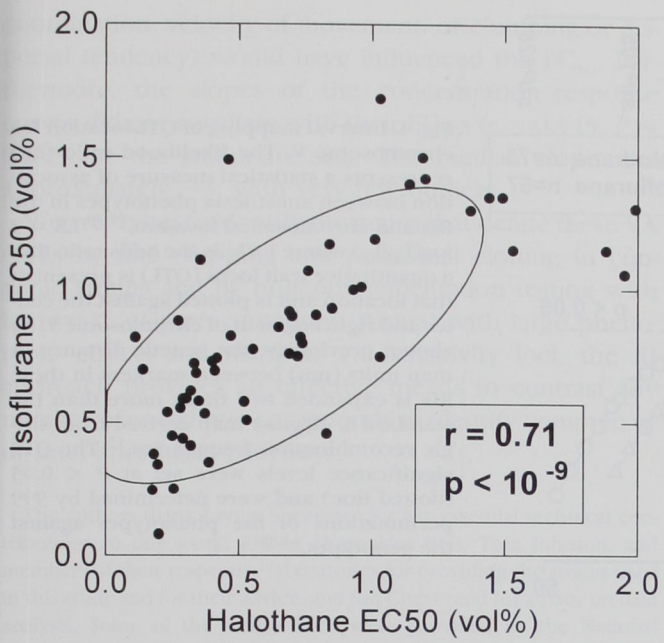

Fig. 2. Correlation of halothane and isoflurane sensitivities. A scatterplot of halothane and isoflurane median effective concentration (EC_{50}) phenotypes (n = 57) for the dispersal end point. A 1-SD confidence ellipse for the data is shown (the thinner the ellipse, the tighter the correlation). The Pearson correlation statistic and its corresponding probability value are inset.

different behavioral end points for halothane: uncoordination and immobility. The similarity of anesthetic mechanisms was addressed at two levels: strain correlations and QTL positions.

As with halothane, the RI strains segregated a wide range of isoflurane EC_{50} phenotypes. The genetic components responsible for creating this variance in the potency of isoflurane against coordinated movement markedly overlapped with those controlling halothane sensitivity in the same assay. The SDP correlation coefficient for either drug, 0.71, is close to the within-strain correlation (0.70) for one drug (isoflurane), suggesting that any lack of correlation between drugs is probably environmental variance and not genetic variance. Predictably, the strongest QTL for halothane and isoflurane mapped to exactly the same genomic location, near *stP6* on chromosome V. The strong SDP correlation suggests that the other QTLs on chromosomes I and II, identified previously by a larger data set for halothane sensitivity,[13] might also be detected by a larger isoflurane data set, although this is not necessarily the case. Conversely, colocalized QTLs clearly do not equate to positive phenotypic correlations. Identical QTLs may result from different genetic interactions among the same genes, and, as such, their phenotypes need not correlate at the strain level. The halothane and isoflurane RI data failed to show such complexity.

Three factors that may limit this study should be emphasized. First, it is important to consider the nature of the RI context. Only loci that have existing DNA sequence polymorphisms in the two parental strains can contribute to differences in phenotype and therefore be detectable as QTLs. Important genes controlling anesthesia may not be detected if they are not different in the parental strains used to make the RIs. Furthermore, any QTLs that are detected by this approach do not necessarily code for the targets of anesthetics. Rather, they may represent molecules upstream, downstream, or parallel to the actual anesthetic targets. Nevertheless, such genes can provide an inroad to the primary anesthetic mechanism. Similarly, although perhaps not central to the primary anesthetic mechanism, an environmental factor (a worm pheromone) modulating anesthetic sensitivity in the dispersal assay may give fundamental insight into anesthetic action.[17] This large component of the environmental variance was discovered *post facto* and was not controlled for in these experiments. However, we are investigating the cause and genetic determinants of this inducible means of resistance to VAs.[17]

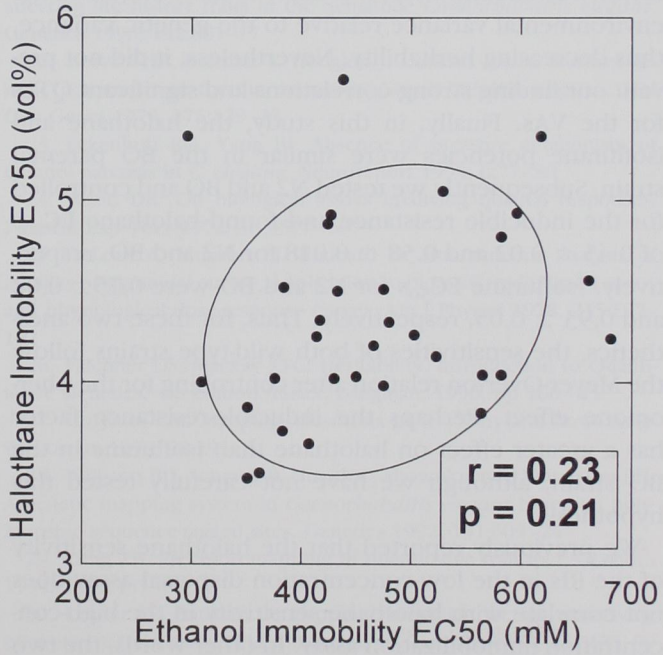

Fig. 3. Correlation of ethanol-and-halothane–induced immobility. A scatterplot of the median effective concentration (EC_{50}) phenotypes for halothane immobilization on agar *versus* ethanol immobilization in liquid (n = 32). A 1-SD confidence ellipse for the data is shown, and the correlation statistics are inset.

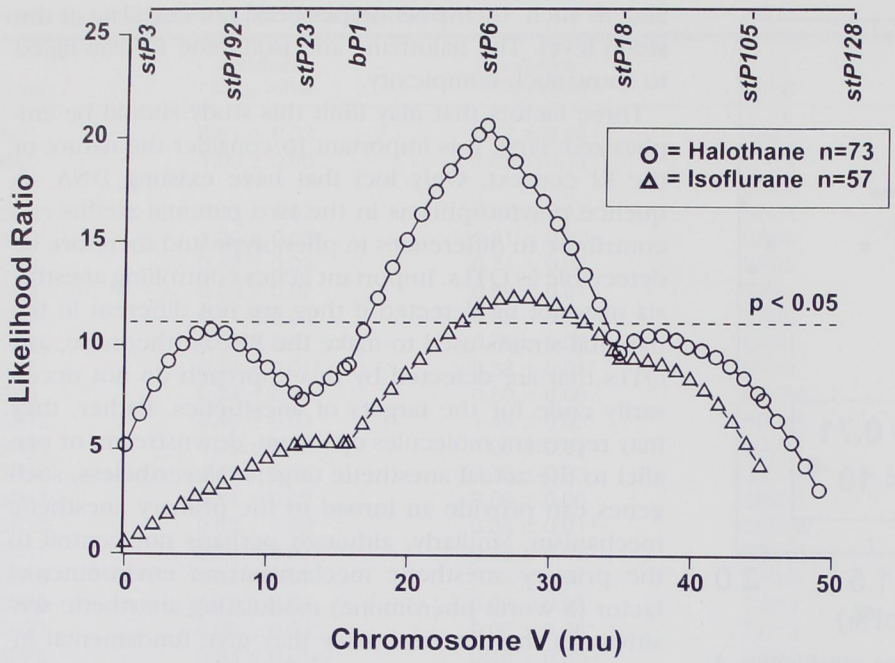

Fig. 4. Interval mapping of QTL location for chromosome V. The likelihood ratio (LR) represents a statistical measure of association between anesthesia phenotypes in the RIs and chromosomal location.[27,30] LR = 2 $\log(L_1/L_0)$ where L_1/L_0 is the odds ratio that a quantitative trait locus (QTL) is present at that location and is plotted against the center and right segment of chromosome V. As shown previously, the genetic distance in map units (mu) between markers in these RIs is expanded two times more than the standard *C. elegans* map derived from single recombination frequencies.[13] The QTL significance levels were set at $P < 0.05$ (dotted line) and were determined by 999 permutations of the phenotypes against the genotypes.[13,31]

Perhaps the anesthetic QTLs modulate sensitivity through this pheromone, or the effect of the QTLs is enhanced or diminished by the pheromone. In terms of heritability, because the effect of this environmental factor was uncontrolled, it is likely to have increased the environmental variance relative to the genetic variance, thus decreasing heritability. Nevertheless, it did not prevent our finding strong correlations and significant QTLs for the VAs. Finally, in this study, the halothane and isoflurane potencies were similar in the BO parental strain. Subsequently we tested N2 and BO and controlled for the inducible resistance and found halothane EC_{50}s of 0.45 ± 0.02 and 0.58 ± 0.018 for N2 and BO, respectively. Isoflurane EC_{50}s for N2 and BO were 0.75± 0.02 and 0.93 ± 0.05, respectively. Thus, for these two anesthetics, the sensitivities of both wild-type strains follow the Meyer-Overton relation after controlling for the pheromone effect. Perhaps the inducible-resistance factor has a greater effect on halothane than isoflurane in the BO strain, although we have not carefully tested this hypothesis.

We previously reported that the halothane sensitivity of the RIs in the low-concentration dispersal assay does not correlate with halothane sensitivity in the high-concentration immobilization assay. In other words, the two end points are controlled by distinct genetic determinants.[13] Here we find that the ethanol immobilization SDP also does not correlate with the halothane or isoflurane SDP from the dispersal assay. Furthermore, the ethanol SDP failed to correlate with the halothane immobility SDP. Although the lack of correlation of halothane and isoflurane uncoordination sensitivities with ethanol immobilization simply may be a result of the very different types of behavioral assays used to compare uncoordination with immobilization, the negative result with halothane immobilization is less likely to result from the end point and more likely to be caused by distinct mechanisms for immobilization of *C. elegans* by ethanol *versus* VAs. The two interacting QTLs mapped for ethanol immobilization did not colocalize with any QTLs found for the two VAs. In contrast to the strong ethanol QTLs found by a mobility assay, previous results for halothane-induced immobility failed to identify any significant loci. Therefore, colocalization of halothane and ethanol immobilization QTLs may exist but may be below the level of sensitivity provided by the 32 strains.

The halothane and isoflurane QTLs identified here by the dispersal assay colocalize with a QTL controlling sensitivity to clinical concentrations of halothane by a completely different assay (a male-mating assay).[13] Thus, three independent lines of experiments point to the chromosomal region near *stP6* as containing a locus or loci controlling sensitivity to clinical concentrations of VAs. Importantly, as noted before, the QTL that maps to *stP6* does not map near loci controlling coordinated movement or mating in the absence of VAs.[13] This result makes it unlikely that behavioral genes unrelated to those controlling VA sensitivity (*e.g.*, genes controlling

coordination, velocity of movement, or clumping or dispersal tendency) would have influenced the EC_{50}. Furthermore, the slopes of the concentration-response curves did not correlate with their EC_{50}s ($r = 0.145$, $P = 0.283$ for the isoflurane set). The median slope was approximately −3, with very few outliers.

We are trying to identify the genes that define these VA QTLs by fine mapping and positional cloning in congenic strains and by noncomplementation testing with known *C. elegans* mutations. Armed with large phenotypic effects and mapped VA sensitivity loci, the RI approach provides an effective means to contrast any number of anesthetic compounds to identify common or distinct anesthesia pathways.

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