Proc. Natl. Acad. Sci. USA
Vol. 94, pp. 8232–8237, July 1997
Pharmacology

Quantitative trait loci controlling halothane sensitivity in *Caenorhabditis elegans*

(inhalational anesthetics/drug resistance/behavioral genetics/theories of anesthesia)

BRUNO VAN SWINDEREN*, DAVID R. SHOOK†, ROBERT H. EBERT‡, VERA A. CHERKASOVA‡, THOMAS E. JOHNSON†, ROBERT J. SHMOOKLER REIS‡, AND C. MICHAEL CROWDER*§

*Departments of Anesthesiology and Molecular Biology/Pharmacology, Washington University School of Medicine, St. Louis, MO 63110; ‡Departments of Medicine and Biochemistry/Molecular Biology, University of Arkansas for Medical Sciences, and the J. L. McClellan Veterans Medical Center, Little Rock, AR 72205; and †Institute for Behavioral Genetics and Department of Environmental, Population, and Organismic Biology, University of Colorado, Boulder, CO 80309

Communicated by Howard A. Nash, National Institute of Mental Health, Bethesda, MD, March 13, 1997 (received for review September 23, 1996)

ABSTRACT **Genetic analysis is an essential tool for defining the molecular mechanisms whereby volatile anesthetics (VA) disrupt nervous system function. However, the degree of natural variation of the genetic determinants of VA sensitivity has not been determined nor have mutagenesis approaches been very successful at isolating significantly resistant mutant strains. Thus, a quantitative genetic approach was taken toward these goals. Recombinant-inbred strains derived from two evolutionarily distinct lineages of the nematode *Caenorhabditis elegans* were tested for sensitivity to clinically relevant concentrations (0.3–0.5 mM) of the VA halothane. The halothane sensitivities of coordinated movement and male mating behavior were highly variant among the recombinant-inbred strains with a range of EC_{50} values of 13- and 4-fold, respectively. Both traits were highly heritable (H^2 = 0.82, 0.87, respectively). Several strains were found to be significantly resistant to halothane when compared with the wild-type strain N2. A major locus or loci mapping to the middle of chromosome V accounted for more than 40% of the phenotypic variance for both traits. Five weaker loci, four of which interact, explained most of the remaining variance. None of the halothane-sensitivity quantitative trait loci significantly affected behavior in the absence of halothane or halothane's potency for *C. elegans* immobilization, which requires 5-fold higher drug concentrations. Thus, the quantitative trait loci are unlikely to result from differences in halothane-independent (native) behavior or differences in halothane metabolism or permeability. Rather, these loci may code for targets and/or downstream effectors of halothane in the *C. elegans* nervous system or for modifiers of such gene products.**

Volatile anesthetics (VAs) produce profound effects at similar concentrations on the behavior of all metazoans so far tested (1–3). However, the molecular mechanism(s) responsible for anesthetic behavioral effects is unknown. Electrophysiological studies have demonstrated that synaptic potentials are significantly more sensitive than axon potentials (4, 5); thus, the site of action of VAs is widely assumed to be the synapse. Indeed, several ligand- and voltage-gated ion channels concentrated at synapses are affected by anesthetic concentrations similar to those required to produce anesthetic behavioral endpoints (6–10). However, which, if any, of these *in vitro* effects is responsible for the behavioral dysfunction is unclear. Given the only modest electrophysiologic specificity of VAs, the lack of binding data to neuronal tissue, and the absence of specific pharmacological inhibitors, genetics is perhaps the only viable approach for identifying molecules actually responsible for anesthesia.

However, genetic control of VA sensitivity, particularly that resulting in anesthetic resistance, has not been widely demonstrated. Rodent strains that were selectively bred for sensitivity to N_2O or to nonanesthetic nervous system depressants express only small, and for the most part statistically insignificant, differences in their sensitivity to VAs (11–15). No loci controlling these small differences in anesthetic sensitivity have been mapped. More success in demonstrating genetic control of VA sensitivity has been achieved in invertebrates. Mutant strains of *Drosophila melanogaster* have been isolated that are significantly hypersensitive (2) or resistant (16) to the VA halothane. The relative differences between wild-type and mutant strains in sensitivity to halothane are 1.2- to 1.5-fold as measured by the median effective halothane concentrations (EC_{50}s).

In the nematode *Caenorhabditis elegans*, mutants that are as much as 3-fold hypersensitive to halothane-induced immobilization along with their genetic supressors have been isolated (17–19), and some of the genes are being positionally cloned (P. Morgan, personal communication). However, the halothane concentration required for immobilization is 12-fold greater than those required for anesthetic endpoints in vertebrates and *Drosophila* (1–3). These halothane concentrations produce a wide range of electrophysiologic and membrane effects on vertebrate neurons not seen at clinically relevant concentrations (1). Thus, the relevance of the gene products identified by these mutations to human anesthetic mechanisms is unclear. While immobilization by VAs occurs at markedly supraclinical concentrations, other *C. elegans* behaviors are abolished by concentrations essentially identical to those required in vertebrates (3). Specifically, coordinated movement, mating, and chemotaxis are rapidly and reversibly abolished with halothane EC_{50}s of approximately 200–400 μM (0.3–0.6 volume % at 20°C). Given their pharmacologic similarity to vertebrate anesthesia, these anesthetic behavioral effects in *C. elegans* may be mediated by homologues of those operant in human anesthetic action.

Whereas mutagenesis has demonstrated that modulators of anesthetic action can be genetically manipulated, the degree that anesthetic sensitivity varies by mutation appears small, and the natural variance of anesthetic sensitivity has not been examined. Thus, a quantitative genetic approach, where the simultaneous variation of multiple genes can be examined, was taken. Using recombinant-inbred strains (RIs) derived from two divergent lineages of *C. elegans*, we examined the variation and heritability of sensitivity to the VA halothane. Disruption of coordinated movement and mating were used as anesthetic

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.

© 1997 by The National Academy of Sciences 0027-8424/97/948232-6$2.00/0
PNAS is available online at http://www.pnas.org.

Abbreviations: QTL, quantitative trait locus; RIs, recombinant inbred strains; VA, volatile anesthetic.

§To whom reprint requests should be addressed.

Downloaded at Palestinian Territory, occupied on November 24, 2021
www.manaraa.com

endpoints. These traits were found to be highly variant, heritable, and their genetic determinants mappable as quantitative trait loci (QTLs).

MATERIALS AND METHODS

Nematode Strains. RIs were produced from two isogenic wild-type *C. elegans* lineages, Bristol-N2 (20) and Bergerac-BO, (21) in three separate crosses, (22, 23) and were inbred to homozygosity (>F15). The strains assayed were chosen at random from these stocks. Males for each strain were obtained by heat-shocking L4 hermaphrodites (24), and male progeny were intercrossed with their hermaphrodite siblings to produce sufficient males for behavioral testing. As expected for strains derived from the impotent BO strain, some strains did not produce potent males, even after multiple attempts. Strains were grown and maintained at 20°C on nutrient growth medium agar plates seeded with *Escherichia coli* strain OP50 (20).

Behavioral Assays. General methods for delivering VAs to *C. elegans* and the potency and time course of action of the VA halothane against coordinated movement, male mating behavior, and gross movement in N2 have been described previously (3). Halothane (Halocarbon Products, Hackensack, NJ) was injected as a liquid into air-tight glass chambers (Corning) containing agar plates for each strain. After the experiment, gas phase halothane concentrations were measured by gas chromatography, and the volume percentage determined by interpolation against standards. All assays were performed at 20–22°C on well fed young adult animals that had not gone through the dauer larvae stage. Concentration/response data were fit by nonlinear regression to estimate EC_{50}s, slopes, and standard errors of the estimate. Statistical comparisons of EC_{50}s were performed by simultaneous curve fitting as described by Waud (25) and as modified by De Lean (26). At least six data points for each strain were used to estimate EC_{50}s. The significance threshold for resistance or hypersensitivity as compared with the wild-type strain N2 was set at 0.05 with Bonferroni correction for the number of strains compared. The number of strains used in our analyses were 73 RIs in the dispersal assay, 31 in the mating assay, and 57 in the immobilization assay along with 10 replicate measurements of the wild-type strains for each assay.

Coordinated movement was scored by the radial dispersal assay as described previously (3) with the following modifications. After washing young adult worms to remove bacteria and suspending them in distilled water, the worms were allowed to sit for as long as 30 min before transferring them to dispersal plates in 10 μl of water. In addition, upon initial dispersal the plates were shaken until the majority of worms moved away from the central clump. These two modifications were made to improve reproducibility among strains but also resulted in higher (0.79 vs. 0.32 vol %) halothane EC_{50}s for N2 than previously reported (3).

Male mating efficiency (24) in halothane for each strain was determined by a modification of the method of Crowder *et al.* (3) by placing two young males and two L4 or young adult *dpy-11*(*e224*) hermaphrodites onto a 1.5-cm well filled with nutrient growth medium agar seeded with OP50 *E. coli*. Sixteen mating pairs per strain were tested simultaneously in a 16-well plate placed in a glass chamber with various halothane concentrations. All males were removed from the wells after a 24-hr mating trial, and the fraction of wells with greater than two cross progeny (allowing for larval contamination while transferring the males) was scored as the mating efficiency (24). Heat shock of some strains consistently failed to produce sufficiently potent males for maintenance of male lines. Therefore, these strains were not used for estimating heritability or mapping mating anesthesia or native mating QTL. The impotent strains are included in the strain distribution pattern for native mating efficiency and are given mating efficiencies of zero.

For immobilization by halothane, hermaphrodites were grown at low density (50–100 worms per plate) on 3.5-cm nutrient growth medium plates seeded with OP50 *E. coli*. Two plates per strain were tested at each halothane concentration after an equilibration time of 2 hr as described previously (3, 17). An animal was counted as immobilized if it did not reverse, advance, or bend more than half of its body during a 10-sec period. Thirty young adult animals were scored per plate, thus 60 per strain, and the average fraction of individuals moving for the two separate counts was plotted against halothane concentration.

Genotyping. TC1 transposon-based sequenced-tagged sites dispersed throughout the *C. elegans* genome (27) were used as dominant markers for the presence of the BO genome at that position. All RIs had been previously genotyped for 27 markers that are dimorphic between BO and N2 (22, 23). The marker coverage is approximately 55% assuming a 5-map unit extension past the outside most markers for each linkage group (23). Recombination frequency between markers and bias in marker genotypes in the RIs have been reported previously (22, 23).

QTL Mapping. ANOVA was used to quantitate the linkage of a marker with a genetic determinant of halothane sensitivity (28). In simple terms for a given marker, the strains were divided into those with either the BO or N2 allele. The two groups were then compared by ANOVA to determine if their mean EC_{50}s significantly differed. This process was repeated for all 27 markers for each trait, and the results are expressed as the F ratio. ANOVAs were performed with the SYSTAT statistical package (Systat, Evanston, IL). A QTL was assigned to a peak marker effect with a threshold α value < 0.05. The genome-wide α value was estimated by two methods. First, the probability of given F ratio was determined assuming normal distribution of the data. The α value then was Bonferroni-corrected for 11 nonredundant linkage clusters among our marker set (22). Thus, an α value of $0.05/11 = 0.0045$ was set as the significance threshold. Secondly, an empirical determination of the probability of a given F ratio was performed as described by Churchill and Doerge (29). Here no assumption of normality is made. The phenotypic and genotypic data are permuted 999 times to determine the probability of a given F ratio occurring by chance at a specific genomic location (comparison-wise probability) and the probability of a given F ratio occurring by chance across the entire genome (experiment-wise probability) (29). Permutations were performed using the QTL CARTOGRAPHER mapping program (North Carolina State University, Raleigh, NC) (30). An experiment-wise $\alpha < 0.05$ was set as the significance threshold. The experiment wise threshold was always more conservative than Bonferroni-corrected F ratios. QTLs significant only by the Bonferroni-correction method are called tentative QTLs, and those significant by both methods are called QTLs.

For the mating anesthesia trait, the concentration/response data for each strain were independently replicated; the replicate data for mating anesthesia were highly repeatable (r_s = 0.71; coefficient of variation = 14%). Thus, a nested ANOVA (28) with the regression equation, phenotype = constant + marker + strains{marker}, was used for mapping mating anesthesia QTLs. However, for the permutation method, the means of the replicate mating anesthesia EC_{50}s were used, because algorithms for permuting nested ANOVAs are not available. Unnested ANOVAs with either mating anesthesia data set gave the same marker peaks as the nested ANOVAs but with smaller F ratios. To avoid type-1 error, nested regression was not used for native mating phenotypes, because the ceiling value of 100% for native mating efficiency artificially decreases the within strain variance (28).

Downloaded at Palestinian Territory, occupied on November 24, 2021

www.manaraa.com

Dispersal and mating anesthesia QTLs that were detectable as genetic interactions between two loci were sought by ANOVA or nested ANOVA, respectively, with the regression equation: EC_{50} = constant + marker1 + marker2 + marker1 × marker2 + [strains{marker1 × marker2}]; [ ] is included only for nested ANOVA. The threshold for significance for the interaction term was set at $\alpha < 0.0009$ ($\alpha < 0.05/55$ possible pairwise tests of 11 independent linkage clusters).

The locations of dispersal and mating anesthesia QTLs relative to the physical markers were estimated by interval mapping (30, 31). Likelihood ratios were determined by interval mapping with an RI self paradigm using 1-map-unit intervals (Zmapqtl model 3, simple interval mapping, QTL CARTOGRAPHER program). The locations of the QTLs (peak likelihood ratio in the interval) as given in Table 1 are relative to the standard *C. elegans* genetic map and were corrected for the amount of map expansion present in that particular interval in these RIs (22, 23).

RESULTS

The genetic control of sensitivity to VAs was examined in *C. elegans* RIs. RIs are made by crossing two parental strains that are polymorphic at multiple loci dispersed throughout their genomes. The F2 or some subsequent generation is then inbred (in the case of *C. elegans*, males are removed and progeny of self-fertilization are propagated) for at least 10 additional generations. Any particular RI strain is composed of a unique mixture of loci derived from one or the other parental allele and, given the degree of inbreeding, is homozygous at every locus. The parents for these RIs were two wild-type strains, *C. elegans* var. Bristol-N2 (20) and *C. elegans* var. Bergerac-BO (21), which are evolutionarily divergent (32). These RIs have proven useful for mapping other quantitative traits (22, 23). The primary phenotypes scored were the sensitivity of coordinated movement (in the dispersal assay) and male mating behavior to the VA halothane (see *Materials and Methods*). We refer to these anesthetic behavioral effects that occur at concentrations and with time courses similar to those required for human anesthesia (3) as dispersal anesthesia or mating anesthesia. As controls, the same strains also were scored for immobilization by halothane, which as discussed above requires 12 times the concentrations needed for vertebrate anesthesia (EC_{50} = 3.5 vol % or 2.5 mM at 20°C) (3, 17), and for native (in the absence of halothane) dispersal and mating efficiency. Loci that affect halothane potency against dispersal and male mating but do not alter the potency of halothane-induced immobilization are unlikely to control halothane metabolism or permeability, and those that do not alter the native behaviors are unlikely to act merely by augmenting a native behavioral defect.

Halothane Sensitivity Is Highly Heritable. The strain distribution patterns for all three anesthetic endpoints and the native behaviors are shown in Fig. 1. The sensitivity of the RI lines to halothane was highly variant for both dispersal and mating anesthesia compared with the parental within-strain variance (Fig. 1 *A* and *B*). The RIs were less variant for halothane paralysis (Fig. 1*C*). The genetic determinants segregating in these strains conferred a 13-fold range in halothane potency for dispersal anesthesia, a 4-fold range for mating anesthesia, and 1.6-fold range for halothane immobilization. Broad-sense heritability ($H^2 = V_g/(V_g+V_e)$) (33) for both dispersal ($H^2 = 0.81$) and mating anesthesia ($H^2 = 0.87$) was quite high; for immobility the heritability was 0.67. The mean of the N2 and BO phenotypic variances ($V_{p(N2+BO)/2} = 0.0204$) provided an estimate of environmental variance, V_e, for dispersal anesthesia while only the N2 phenotypic variance ($V_{pN2} = 0.0022$) was used for mating heritability because BO males do not mate. The genetic variance, V_g, for RIs = $\frac{1}{2}(V_p - V_e)$ (34). The range (D) and variance (V_p) of RI phenotypes predict a minimum of four QTLs for both dispersal and male mating anesthesia as estimated by Taylor's modification of Wright's formula ($D^2/4V_p$) (35). For halothane-induced paralysis, at least six QTLs are predicted. The rank order of the immobilization EC_{50}s did not correlate with that for mating or dispersal anesthesia (mating: $r_s = 0.13$, $P = 0.5$; dispersal: $r_s = 0.13$, $P = 0.5$, Spearman's nonparametric rank correlation). Thus, the genetic components responsible for the variance in mating anesthesia and dispersal anesthesia are dissimilar to those for halothane immobilization.

Not unexpectedly, given the poor native male mating efficiency of the BO parent, the RIs were also variant for male mating in the absence of halothane (Fig. 1*D*); the partly bimodal distribution of these natural mating efficiencies suggests one major gene effect along with some number of minor genes. For native dispersal as opposed to dispersal anesthesia, the majority of the variance is environmental; however, the heritability is still substantial at 0.377.

Halothane Resistance. For both dispersal ($n = 4$) and mating anesthesia ($n = 6$), significant halothane resistance relative to the wild-type strain N2 was a common phenotype (Fig. 1 *A* and *B*). Significant resistance to halothane-induced immobilization was also common (Fig. 1*C*). In contrast, over 500,000 mutagenized genomes have been screened for the halothane-resistant phenotype without isolating a single halothane-resistant strain (P. Morgan, personal communication). Interestingly, the mean halothane immobility EC_{50} of the RIs was significantly greater than either parental mean ($P < 0.01$). This phenotypic shift along with the rarity of the resistance in mutagenesis screens suggests that the resistance to these supraclinical halothane concentrations is produced by genetic interaction (36), in this case among novel allelic combinations of BO and N2 genes. Significant halothane hypersensitivity was also commonly seen for all three traits.

QTLs for Halothane Sensitivity. The loci controlling the large genetic variances in halothane potency in the RIs were mapped by testing for linkage to sequence-tagged site markers dimorphic between BO and N2 (27). The results of the linkage

Table 1. QTLs for halothane sensitivity

Nearest marker	Assay	Mean EC_{50} RIs, vol %*		F ratio	P value†	% V_p	Location, map units‡
		BO allele	N2 allele				
stP124 (*IC*)	Dispersal	1.12 ± 0.12	0.69 ± 0.05	16.8	0.0001	22	0.4
stP6 (*VR*)	Dispersal	0.58 ± 0.05	1.01 ± 0.07	23.2	0.000008	42	5.9
stP18 (*VR*)	Mating	0.46 ± 0.02	0.66 ± 0.02	53.7	0.000000003	48	9.7
stP124 (*IC*) × *stP98* (*IIC*)	Mating	NA	NA	23.4	0.00003	11	NA
bP1 (*VC*) × *stP129* (*XC*)	Mating	NA	NA	32.9	0.000003	16	NA

*Mean ± SEM of the EC_{50}s for all strains with either the BO or the N2 allele at that marker.
†Bonferroni-corrected significance thresholds are 0.05/11 = 0.0045 for single QTL and 0.05/55 = 0.0009 for interacting QTLs. Only single QTLs that are also significant at the 0.05 level by experiment-wise permutation are listed. The F ratios/P values for the interacting QTLs are for the interaction term of the regression equation.
‡Location of the peak likelihood ratio by interval mapping relative to the standard *C. elegans* genetic map.
NA, not available.

Downloaded at Palestinian Territory, occupied on November 24, 2021

www.manaraa.com

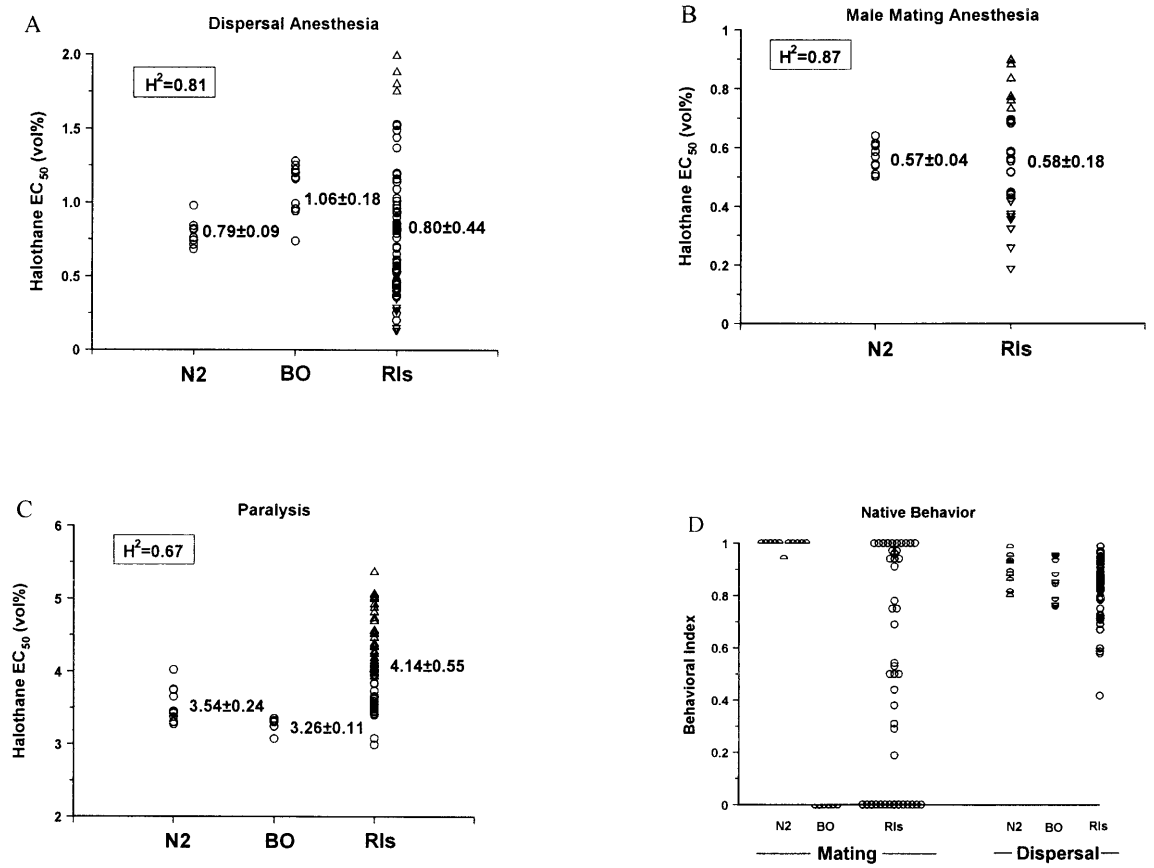

FIG. 1. Strain distribution pattern for halothane potency in the RIs and their parents. (*A*) Dispersal in halothane. EC_{50}s for each RI (n = 73), the N2 (n = 10) and the BO parents (n = 10) and their group means and SD are given. H^2 = broad sense heritability. △, halothane-resistant RIs ($P < 0.05/73 = 0.0007$): SR255, SR2, TJ202, TJ207. ▽, halothane-hypersensitive RIs: TJ103, TJ120, TJ147, SR109, TJ216, SR269, and TJ242. (*B*), Male mating efficiency in halothane. Mean EC_{50}s for each RI ($n = 31 \times 2$ trials) and for the N2 parental strain (n = 10). The BO males did not mate well enough to be assayed. △ ($P < 0.05/31 = 0.0016$): SR83, SR255, SR119, SR58, SR25, and SR28. ▽, SR62, SR42, SR117, TJ223, and SR226. (*C*) Halothane-induced immobility. EC_{50}s are plotted for each RI (n = 57), N2 (n = 10), and BO (n = 5). △, RIs significantly more resistant than N2 ($P < 0.05/57 = 0.0009$). (*D*) Native mating in the absence of halothane. Mating efficiencies and dispersal indices are plotted for N2 ⌓, BO ⌔, and RIs ○.

analysis for dispersal and mating anesthesia as well as for halothane immobilization are shown in Fig. 2. Loci that are significant after Bonferroni correction of P values from marker regression are indicated by asterisks. Loci that are also significant at the 0.05 genome-wide level after permutation of the phenotypic data (29) are considered unequivocal QTL and are listed in Table 1 along with their interval locations. A powerful effect on halothane potency for both dispersal and mating anesthesia is linked to markers in the central region of chromosome V. The QTL for each trait peaks at adjacent markers and explains 42% of the phenotypic variance for dispersal anesthesia and 48% for mating anesthesia. An additional QTL linked to *stP124* on chromosome I had a major effect on dispersal anesthesia. Two adjacent tentative QTLs on chromosome II, *stP100* and *stP196*, had strong effects on dispersal and mating anesthesia, respectively. A tentative QTL for both mating and dispersal anesthesia maps near *stP44* at the center of chromosome IV. Finally, tentative QTLs on the left arm of chromosome V (*stP3*) and the center of the X chromosome (*stP129*) were mating specific. No marker effect reached significance for halothane paralysis although the F ratio at *stP100* was near significance (F = 7.94, P = 0.007).

Genetic Interactions Between Loci. Genetic determinants can exert their effects independently or confer phenotypic variance by interaction with other loci. Halothane-sensitivity QTLs that exert their effects primarily by gene interaction were sought by pair-wise marker regression. Two significant interacting pairs of loci were identified for male mating anesthesia (Table 1). One mating anesthesia interaction involved chromosomes II (*stP98*) and I at the *stP124* marker, which was implicated in dispersal anesthesia. The locus linked to the *stP98* marker, while not significant for mating anesthesia when considered alone, genetically interacts to influence halothane potency significantly ($P < 0.0009$). The other highly significantly interacting locus pair mapped to the middle of chromosomes V and X. For dispersal anesthesia, two near-significant interactions (*stP124* × *stP18*, P = 0.003; *stP100* × *stP18*, P = 0.001) involve loci already implicated as individual QTL but as interactions are not significant after Bonferroni correction. No significant or near-significant two-way interactions were detected for halothane paralysis.

QTL for Native Behavior. A genome wide scan for loci that affect mating and dispersal behavior itself in the absence of anesthetic identified a single QTL on the left arm of chromosome V linked to *stP3* (Fig. 3). This QTL significantly influenced both dispersal and mating efficiency but maps to a region of chromosome V distinct from the dispersal anesthesia (12 map units away) and the mating anesthesia (16 map units away) QTLs. To identify loci that render males completely unable to mate as is the case for the BO parent, 14 RIs that consistently failed to produce potent males were included in a separate analysis. Here, strains were categorized only as impotent vs. potent, and *stP100* (II L) was found to affect significantly ($P < 0.0005$) native mating efficiency. Thus, with the exception of the tentative mating anesthesia QTL at *stP3*, variations in native behavior do not account for the QTLs controlling mating or dispersal anesthesia.

Downloaded at Palestinian Territory, occupied on November 24, 2021

www.manaraa.com

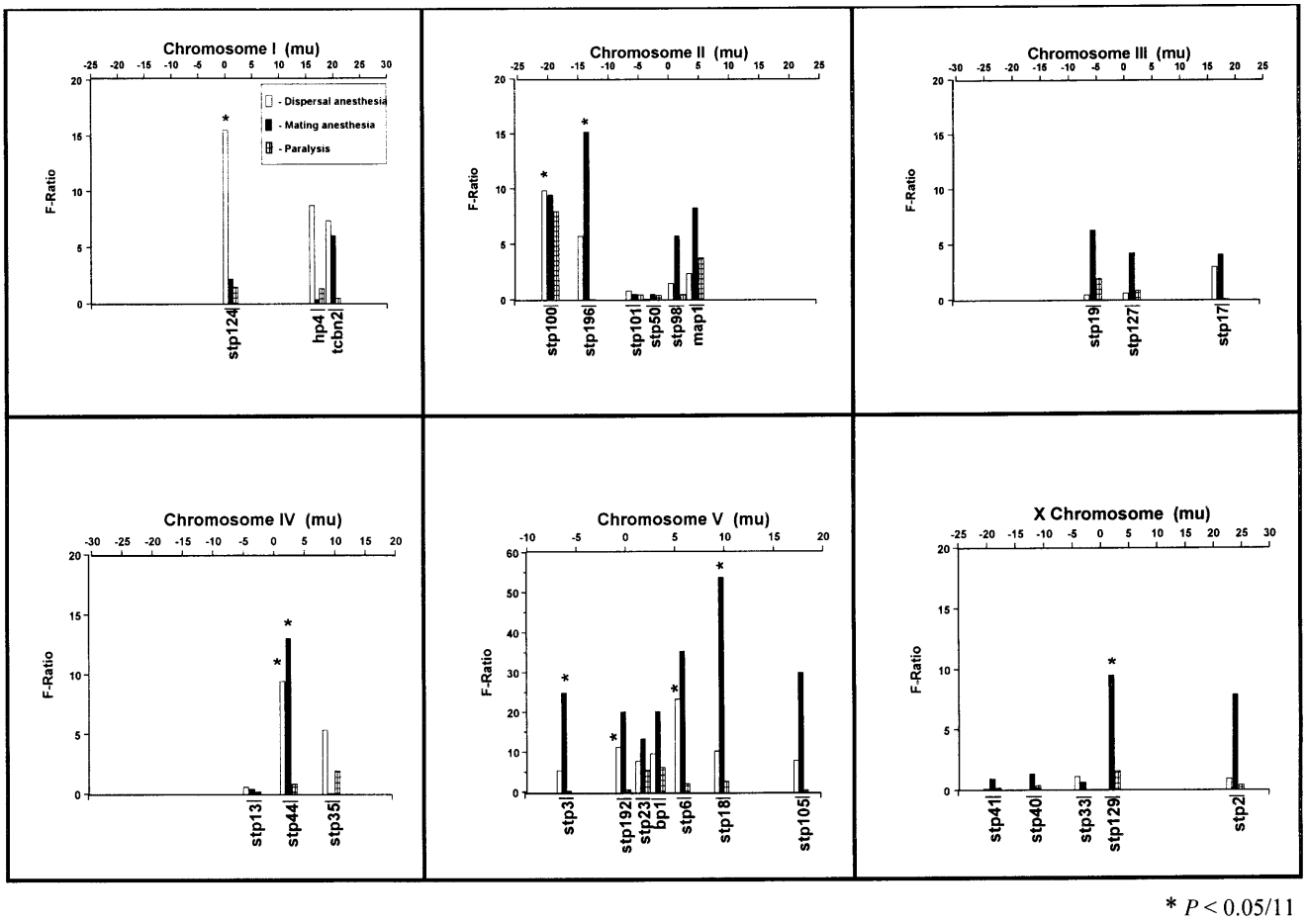

FIG. 2. Genome scan for dispersal anesthesia, mating anesthesia, and halothane-immobility QTLs. ANOVAs were performed at each marker for the six *C. elegans* linkage groups with three different sets of dependent variables: halothane EC_{50}s against dispersal (open bars), against male mating (solid bars), and against movement (crosshatched bars). The genetic markers are on the *x* axis along with their location relative to the standard *C. elegans* linkage map. *F* ratios are given on the vertical axis. Note that the vertical scale on chromosome V is compressed. * indicates significance at $P < 0.0045$.

DISCUSSION

Volatile anesthetics affect the function of multiple important nervous system proteins (1). Even when effects are limited to those occurring at relevant concentrations, a number of potential mediators of general anesthesia remain. Thus, behavioral disruption by volatile anesthetics could be due to a summation of effects at multiple targets, and genetic control of anesthetic sensitivity might fruitfully be approached as a polygenic trait. In this work, we demonstrate that sensitivity to clinically relevant concentrations of halothane is heritable and genetically tractable as a polygenic trait and that natural variation can generate large differences in the anesthetic sensitivity of *C. elegans*.

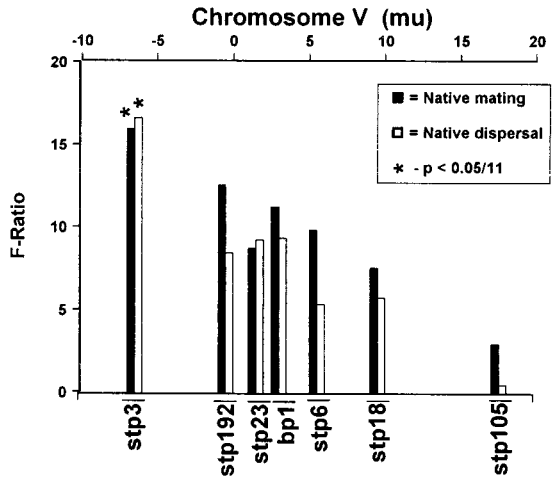

FIG. 3. Native (in the absence of halothane) dispersal and mating QTL. The dispersal index (fraction of animals dispersed into the bacterial ring after 40 min) or mating efficiency (fraction of mating trials resulting in crossprogeny) of the RIs in the absence of halothane was mapped against all markers on the six chromosomes. Significant QTLs were only found on chromosome V.

Compared with previous efforts, the frequency and level of halothane resistance seen in the RIs for both dispersal and mating anesthesia and immobility were unexpected. Large mutagenesis screens (>500,000 genomes) for resistance to halothane-induced immobility have been done without isolating a single resistant strain (P. Morgan, personal communication). We have screened approximately 20,000 genomes for resistance to clinical concentrations of halothane without finding strains that are clearly resistant. The relative ease of finding resistance when halothane action is approached as a polygenic trait has at least two reasonable explanations. First, halothane could have multiple targets so that mutation of any single target does not produce a discernible change in drug sensitivity (i.e., genetic redundancy). Second, the genetic determinants of halothane sensitivity could be essential for viability or normal behavior (i.e., pleiotropic effects). If so, mutagenesis screens that most commonly isolate null mutants might not easily find viable halothane-resistant strains. How-

Downloaded at Palestinian Territory, occupied on November 24, 2021

www.manaraa.com

ever for halothane-induced paralysis, if a single missense mutation could produce halothane resistance without complete loss of gene function, such a mutant likely would have been isolated in a 500,000-genome screen. Thus, we favor the former explanation that relatively high-level halothane resistance may require simultaneous sequence changes in more than one gene.

While the identified QTLs confer large changes in anesthetic sensitivity, they are unlikely to represent all of the genetic determinants of halothane sensitivity in *C. elegans* for a number of reasons. Most importantly, some genes may not be variant in the N2 and BO parental strains. The average sequence divergence between N2 and BO has not been accurately estimated but is probably less than 0.5% (37); thus, many genes may not have relevant polymorphisms. Construction of additional RIs from other parental strains as well as larger scale mutagenesis screens seems warranted. For identifying the QTLs responsible for the large variance in halothane sensitivity in these RIs, the sensitivity of this study appears to be high, particularly for dispersal anesthesia. In a regression equation including each QTL, 80% of the genetic variance for dispersal anesthesia is accounted for by the QTLs at *stP124* and *stP6*; for mating anesthesia, 54% of V_g was explained. However, improving genomic coverage from the 55% provided by the existing markers may identify additional loci. Shotgun cloning of the approximately 400 polymorphic TC1 transposons in conjuction with the *C. elegans* genome sequencing project eventually will provide dense marker coverage (38). If present, additional small effect QTLs or interacting QTLs will require a much larger sample size to reach detection.

Some of the QTLs identified in this study have such large effects on anesthetic sensitivity that they may be suitable for positional cloning. The primary prerequisites for cloning these loci are: (*i*) the N2 allele must be dominant or semidominant to the BO allele because the N2 genome has been cloned by the *C. elegans* genome project; (*ii*) a clonable locus must confer a large difference in halothane sensitivity when only it varies; and (*iii*) the recombination distance of the locus from flanking markers must be obtained with good precision. Toward this aim, we have begun to isolate each QTL in congenic strains. For chromosome V QTL(s), the halothane resistant strain SR255, which has a halothane EC_{50} of 1.88 ± 0.15 volume % for dispersal anesthesia and 0.97 ± 0.01 volume % for mating anesthesia, was crossed with the halothane hypersensitive strain TJ299, which has the respective EC_{50}s of 0.27 ± 0.05 and 0.42 ± 0.02. SR255 has an identical genotype to TJ299 except for the seven markers on chromosome V where SR255 is N2-like and TJ299 is BO-like. The halothane EC_{50}s of the F1 progeny were 0.7 volume % for mating anesthesia and 1.35 volume % against dispersal indicating semidominance of the halothane resistance-conferring N2 allele. Mapping of the mating anesthesia trait in the first 25 congenic strains found a single locus on chromosome V peaking at *stP6* with a significant LOD score of 3.9. Those strains with the N2 allele at *stP6* have a mean EC_{50} 2.0-fold greater than those with the BO allele. These results are strong confirmation of the QTL mapped in the RIs to the *stP6–stP18* interval. With additional strains to improve mapping precision, the prerequisites for molecular characterization of a locus that controls sensitivity to clinically relevant concentrations of halothane should be met.

We would like to thank Jim Cheverud for helpful advice in the analysis of our data and for critical reading of the manuscript. We also thank Laynie Shebester for her important technical contributions to this work. This work was supported by a Foundation for Anesthesia Education and Research Ohmeda Young Investigator Award to C.M.C. and National Institutes of Health Grants RO1AG8322 and RO1AG10248 to T.E.J. and RO1AG09413 to R.J.S.R.

1. Franks, N. P. & Lieb, W. R. (1994) *Br. J. Anaesth.* **71,** 607–614.
2. Campbell, D. B. & Nash, H. A. (1994) *Proc. Natl. Acad. Sci. USA* **91,** 2135–2139.
3. Crowder, C. M., Shebester, L. D. & Schedl, T. (1996) *Anesthesiology* **85,** 901–912.
4. Larrabe, M. G. & Posternak, J. M. (1952) *J. Neurophysiol.* **15,** 91–114.
5. Pocock, G. & Richards, C. D. (1993) *Br. J. Anaesth.* **71,** 134–147.
6. Mody, I., Tanelian, D. L. & MacIver, M. B. (1991) *Brain Res.* **538,** 319–323.
7. Jones, M. V., Brooks, P. A. & Harrison, N. L. (1992) *J. Physiol.* **449,** 279–293.
8. Herrington, J., Stern, R. C., Evers, A. S. & Lingle, C. J. (1991) *J. Neurosci.* **11,** 2226–2240.
9. Takenoshita, M. & Steinbach, J. H. (1991) *J. Neurosci.* **11,** 1404–1412.
10. Franks, N. P. & Lieb, W. R. (1988) *Nature (London)* **333,** 662–664.
11. Koblin, D. D., Dong, D. E., Deady, J. E. & Eger, E. I. (1980) *Anesthesiology* **56,** 18–24.
12. Baker, R., Melchior, C. & Deitrich, R. (1980) *Pharmacol. Biochem. Behav.* **12,** 691–695.
13. Koblin, D. D. & Deady, J. E. (1981) *Br. J. Anaesth.* **53,** 5–10.
14. McCrae, A. F., Gallaher, E. J., Winter, P. M. & Firestone, L. L. (1993) *Anesth. Analg.* **76,** 1313–1317.
15. Deitrich, R. A., Draski, L. J. & Baker, R. C. (1994) *Pharmacol. Biochem. Behav.* **47,** 721–725.
16. Dapkus, D., Ramirez, S. & Murray, M. J. (1996) *Anesth. Analg.* **83,** 147–155.
17. Morgan, P. G. & Cascorbi, H. F. (1985) *Anesthesiology* **62,** 738–744.
18. Morgan, P. G., Sedensky, M. & Meneely, P. M. (1990) *Proc. Natl. Acad. Sci. USA* **87,** 2965–2969.
19. Morgan, P. G. & Sedensky, M. (1994) *Anesthesiology* **81,** 888–898.
20. Brenner, S. (1974) *Genetics* **77,** 71–94.
21. Nigon, V. (1949) *Ann. Nat. Zool. Ser.* **11,** 1–132.
22. Ebert, R. H., Cherkasova, V. A., Dennis, R. A., Wu, J. H., Ryggles, S., Perrin, T. E. & Shmookler Reis, R. J. (1993) *Genetics* **135,** 1003–1010.
23. Shook, D. R., Brooks, A. & Johnson, T. E. (1996) *Genetics* **142,** 801–817.
24. Hodgkin, J. (1983) *Genetics* **103,** 43–64.
25. Waud, D. R. (1972) *J. Pharmol. Exp. Ther.* **183,** 577–607.
26. De Lean, A., Munson, P. J. & Robbard, D. (1978) *Am. J. Physiol.* **235,** E97–E102.
27. Williams, B. D., Schrank, B., Huynh, C., Shownkeen, R. & Waterston, R. H. (1992) *Genetics* **131,** 609–624.
28. Sokal, R. R. & Rohlf, F. J. (1981) in *Biometry* (Freeman, New York), pp. 208–320.
29. Churchill, G. A. & Doerge, R. W. (1994) *Genetics* **138,** 963–971.
30. Zeng, Z.-B. (1994) *Genetics* **138,** 1457–1468.
31. Lander, E. S. & Botstein, D. (1989) *Genetics* **121,** 185–199.
32. Egilmez, N. K., Ebert, R. H. & Shmookler Reis, R. J. (1995) *J. Mol. Evol.* **40,** 372–381.
33. Falconer, D. S. (1989) in *Introduction to Quantitative Genetics* (Longman, Essex, UK), pp. 125–133.
34. Hegman, J. P. & Possidente, B. (1981) *Behav. Genet.* **11,** 103–114.
35. Taylor, B. A. (1976) *Genetics* **83,** 373–377.
36. Templeton, A. R. (1980) *Genetics* **94,** 1011–1038.
37. Thomas, W. K. & Wilson, A. C. (1991) *Genetics* **128,** 269–279.
38. Korswagen, H. C., Durbin, R. M., Smits, M. T. & Plasterk, R. H. A. (1996) *Proc. Natl. Acad. Sci. USA* **93,** 14680–14685.

Downloaded at Palestinian Territory, occupied on November 24, 2021

www.manaraa.com