

Title: GENETIC VARIATION OF ANESTHETIC RESPONSE IN *C.elegans*

Authors: P. G. Morgan, M.D.⁽¹⁾, M. M. Sedensky, M.D.⁽¹⁾,
H. F. Cascorbi, M.D., Ph.D.⁽¹⁾ and P. M. Meneely, Ph.D.⁽²⁾

Affiliation: ⁽¹⁾Department of Anesthesia, Case Western Reserve University,
University Hospitals of Cleveland, Cleveland, Ohio 44106 and
⁽²⁾Fred Hutchinson Cancer Research Center, Seattle, Washington 98104.

INTRODUCTION: We are attempting to understand the mechanism of action of volatile anesthetics by means of a simple animal model—the nematode, *Caenorhabditis elegans* (C.e.). We are studying the effect of genetic variation on sensitivity to a wide range of anesthetics. We have previously shown that the wild type strain (N₂) of C.e. responds to volatile anesthetics like more complex animals⁽¹⁾. In our earlier work we also found a mutant with altered anesthetic sensitivity and thus began the analyses necessary to isolate gene products that control anesthetic response. This strain was identified as a previously known mutant, *unc-79*⁽²⁾. We have now identified a second gene, *unc-80*, which also causes altered sensitivities to several anesthetics. We present here additional data describing the response of N₂ and *unc-79* to diethylether and the very lipid soluble agent, thiomethoxyflurane. In addition, we describe the responses of *unc-80* and a strain containing both mutations, *unc-79* and *unc-80*, to nine anesthetics. We also examine the relationship of the potencies to the oil gas partition coefficients (O/G_a) for these anesthetics.

MATERIALS AND METHODS:

Nematodes: C.e. strains N₂ and *unc-80* were obtained from the *Caenorhabditis* Genetics Center in Columbia, Missouri. *unc-79* was generated as previously described⁽¹⁾.

Cultures: Cultures were prepared as previously described⁽¹⁾ except that we now prepare the growth agar without the addition of uracil. This allows for a thinner lawn of E.coli on the plates. Under these conditions the nematodes are more easily visualized and move more vigorously.

Dose-Response Curves: Dose-response studies were performed as previously described.⁽¹⁾ Each study consisted of at least twenty different concentrations of anesthetic, with at least fifty individuals counted at each concentration. Data were analyzed by the methods described by Waud⁽³⁾.

RESULTS: We studied the quantitative response of four strains to eight anesthetics. The mean ED50s with standard errors are presented in the table. As previously noted with N₂ and *unc-79* (labeled HS1 in our previous report), the ED50s for *unc-80* and the double mutant *unc-79;unc-80* tended to increase as the (O/G_a)s of the anesthetics decreased. The responses fell into four groups.

1). For the highly lipid soluble anesthetics (thiomethoxyflurane [TMOF], methoxyflurane [MOF], chloroform [CH], halothane [H]), *unc-79*, *unc-80*, and the double mutant *unc-79;unc-80* were all hypersensitive compared to N₂. *unc-79* was consistently more sensitive than *unc-80*, while the double mutant was very similar to *unc-79* in these anesthetics.

2). For enflurane (E) and flurothyl (FLR), *unc-79* and *unc-80* were resistant compared to N₂, *unc-79* being more resistant than *unc-80*. The double mutant had an ED50 similar to N₂ in both cases.

3). For isoflurane (ISO) and fluroxone (FLX), *unc-79* and *unc-80* showed a small increase in sensitivity compared to N₂. The double mutant showed a slight increase in sensitivity compared to either single mutant.

4). In diethylether (DE), *unc-79*, *unc-80* and *unc-79;unc-80* all showed equal and moderate increases in sensitivity.

DISCUSSION: All three mutant strains showed large increases in sensitivities compared to N₂ when exposed to the very lipid soluble anesthetics. In contrast any differences seen in the less lipid soluble anesthetics were small. The ln-ln plot of potencies vs. (O/G_a)s for these anesthetics closely approximates a straight line with slope -1 for N₂. The Meyer-Overton model of anesthesia depends on this relationship. However, in each of the mutant strains there is a discontinuity between enflurane (O/G_a = 98) and halothane (O/G_a = 224). We interpret the alteration in sensitivities as indicative of an alteration at the locus of action of volatile anesthetics.

REFERENCES:

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	N ₂	<i>unc-79</i>	<i>unc-80</i>	<i>unc-79;unc-80</i>
TMOF	0.29 ±0.04	0.09 ±0.03	0.15 ±0.03	0.08 ±0.05
MOF	0.58 ±0.02	0.28 ±0.05	0.46 ±0.03	0.25 ±0.10
CH	1.47 ±0.02	0.50 ±0.03	0.80 ±0.02	0.54 ±0.03
H	3.18 ±0.04	0.98 ±0.02	1.20 ±0.02	0.72 ±0.02
E	5.89 ±0.08	6.24 ±0.07	6.06 ±0.07	5.82 ±0.07
ISO	7.18 ±0.07	6.67 ±0.08	6.14 ±0.07	5.84 ±0.07
DE	7.53 ±0.07	5.70 ±0.06	5.84 ±0.06	5.60 ±0.06
FLX	10.8 ±0.07	10.1 ±0.07	10.4 ±0.07	9.9 ±0.07
FLR	14.3 ±0.10	15.9 ±0.11	14.9 ±0.10	14.5 ±0.08

Legend: ED50s ± SE for four strains of *C.elegans* in nine anesthetics.
For abbreviations, see text.