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Tail Clamp Responses in Stomatin Knockout Mice Compared with Mobility Assays in Caenorhabditis elegans *during Exposure to Diethyl Ether, Halothane, and Isoflurane*

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Background: **The gene *unc-1* plays a central role in determining volatile anesthetic sensitivity in *Caenorhabditis elegans*. Because different *unc-1* alleles cause strikingly different phenotypes in different volatile anesthetics, the UNC-1 protein is a candidate to directly interact with volatile anesthetics. UNC-1 is a close homologue of the mammalian protein *stomatin*, for which a mouse knockout was recently constructed. Because the *stomatin* gene is expressed in dorsal root ganglion cells, the authors hypothesized that the knockout would have an effect on anesthetic sensitivity in mice similar to that seen in nematodes.**

Methods: **Mice were placed in semiclosed chambers and exposed to continuous flows of diethyl ether, halothane, or isoflurane in air. Using lack of response to tail clamp as an endpoint, the authors determined the EC_{50}s for the knockout strain compared with the nonmutated parental strain. They compared the differences seen in the mouse strains with the differences seen in the nematode strains.**

Results: **Stomatin-deficient mice had a 12% increase in sensitivity to diethyl ether but no significant change in sensitivity to halothane or isoflurane compared with wild type. No defect in locomotion was noted in the mutant mouse.**

Conclusions: **Nematodes and mice with deletions of the *stomatin* gene both have increased sensitivity to diethyl ether. Neither nematodes nor mice with *stomatin* deficiencies have significantly altered sensitivity to isoflurane or halothane. The effects of *stomatin* deficiency cross phylogenetic boundaries and support the importance of this protein in anesthetic response and the use of *C. elegans* as a model for anesthetic action in mammals.**

THE nematode *Caenorhabditis elegans* can potentially serve as an excellent model organism for understanding fundamental cellular processes that are operative in mammalian cells.[1,2] However, its usefulness as a model for unraveling the mechanism of action of volatile anesthetics in mammals has been questioned.[3,4] This uncertainty has been primarily the result of two points. First, the dose required to immobilize *C. elegans* is severalfold higher than that required for the minimum alveolar concentration (MAC) in mammals.[5] Second, it is unclear whether even similar anesthetic endpoints in different organisms are caused by effects on similar physiologic functions. As a result, it would be useful to determine whether mutations in genes othologous to those that alter anesthetic sensitivity in nematodes also affect sensitivity in mammals.

We have previously reported a potential parallel between findings in *C. elegans* and anesthetic sensitivity in human children.[6,7] This previous report focused on the effects of mitochondrial dysfunction on anesthetic sensitivity. A second group of several genes, which does not obviously involve mitochondrial function, also controls anesthetic sensitivity in *C. elegans*.[5,8,9] One member of this group, the gene *unc-1*, strongly affects the behavior of the nematode in volatile anesthetics. Loss of function mutations in *unc-1* specifically increase sensitivity to diethyl ether, whereas gain of function mutations in the same gene increase sensitivity to a wide variety of volatile anesthetics.[9] *unc-1* encodes the nematode homolog of the protein stomatin. Antibody staining and mutant rescue with florescent fusion proteins have shown that *unc-1* is primarily expressed in the nervous system of *C. elegans* and localizes to microdomains called lipid rafts within the cell membrane.[9–11] In mammals, stomatins are expressed in multiple tissues, including the nervous system; their function in neuronal tissue is under active investigation.[12,13] There are multiple homologs of stomatin in both mouse (5) and *C. elegans* (10).

In mice, stomatin is expressed in the sensory neurons of the dorsal root ganglion.[14] Fricke *et al.*[15] have also shown that stomatin is expressed in the trigeminal neurons of rats. Stewart *et al.*[16] have shown that patients with stomatin deficiencies have severe neurologic deficits. Other stomatin-like proteins have been shown to be expressed in the mouse brain. These proteins are most closely similar to another stomatin-like protein in nematodes, UNC-24. Mutations in both *unc-1* and *unc-24* genes cause specific increases in sensitivity to diethyl

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ether. We have shown that in animals with *unc-24* mutations, the UNC-1 protein does not leave the perinuclear area of neurons. Under normal conditions, UNC-1 is found in a punctuate pattern in the cell membrane and expressed only in the nervous system and the four vulva muscles.[10]

Recently, a mouse strain was created in which the *stomatin* gene is knocked out.[17] This animal provides the opportunity to rigorously assess whether anesthetic sensitivity is altered in a mammal by a specific molecular defect uncovered in the nematode. It also allows us to determine whether anesthetic immobility in the nematode correlates with a well-accepted anesthetic endpoint in mammals. Using the tail clamp assay, we have measured the anesthetic sensitivity of mice in which the mouse *stomatin* has been eliminated. We find that the behavior of this animal is exactly analogous to that seen in nematodes, using immobility as an endpoint.

Materials and Methods

Mouse Stomatin Knockout

All studies with mice were approved by the Institutional Animal Care and Use Committee at the Case School of Medicine (Cleveland, Ohio). Narla Mohandas, Ph.D. (New York Blood Center, New York, New York), provided the stomatin knockout mice. The strain contains a 637–base pair deletion of the *stomatin* gene (also known as the 7.2b gene, SwissProt P54116) including the promoter and part of the first exon. The isolation of the strain is described in Zhu *et al.*[17] The knockout strain was rederived at our institution by isolating embryos and crossing the mutation into a 129/J background. All measurements in mice were performed in the knockout strain and in the background strain from which it had been isolated. A small piece of tissue was isolated from the tail of each mouse 3–4 weeks before the anesthetic exposure. DNA was isolated from the tissue and tested for the presence or absence of the mutation using polymerase chain reaction as described.[17]

Nematode Techniques

Immobility was used as the anesthetic endpoint. The techniques for determining the anesthetic sensitivity in *C. elegans* have been previously published.[5,8] The conventions for *C. elegans* nomenclature are followed throughout.[18–20] Standard techniques were used for growing and maintaining cultures of *C. elegans*.[19]

The *unc-1* allele *fc53* was isolated in our laboratory after mutagenesis of N2 with ethylmethanesulfonate.[10,21] The canonical allele of *unc-1(e580)* was obtained from the *Caenorhabditis* Genetics Center (Minneapolis, Minnesota). The UNC-1 protein (285 amino acids, SwissProt Q21190, X chromosome) has 285 amino acids and is 52% identical and 87% similar to the mouse stomatin (284 amino acids, SwissProt P54116, chromosome 2). The *e580* mutation results in a missense mutation leading to an amino acid substitution (E188V). The *fc53* mutation is a 13-bp deletion in exon 4 of the *unc-1* gene which results in a truncated message and no discernible protein on Western blots. Of the stomatin-like proteins in the mouse, stomatin is the most closely related to the nematode stomatin UNC-1.

Anesthetic Exposure

Mice were anesthetized similar to the method described by Sonner *et al.*[22] and by Quasha *et al.*[23] In brief, spontaneously breathing animals were exposed to a volatile anesthetic under conditions in which the rectal temperature was maintained at 37°C. Mice were placed in semiclosed chambers with an entrance port and exhaust port for anesthetic gases and a small opening for the tail of the mouse. The mice were then exposed to continuous flows of diethyl ether, halothane, or isoflurane in humidified air. Mice were not restrained other than their tails were held in place through an opening in the chamber by a small piece of tape adhering to the tail. Clamping of the tail was done first at the most distal part of the tail and then moved proximal for subsequent measurements. Anesthetic concentration was increased stepwise by 0.2% after equilibration times as described previously.[22,23] After approximate doses were established for each anesthetic, concentrations were changed by 0.1% during each change. Each animal was exposed to volatile anesthetics only once. Anesthetics were studied in groups, *i.e.*, studies with halothane were performed first, followed by isoflurane and finally diethyl ether. Anesthetic concentrations were determined by sampling gases at the exhaust port with a glass syringe and determining concentrations by gas chromatography. Because the animals were not intubated, end-tidal concentrations were not determined.

Using lack of response to tail clamp as an endpoint, we determined the EC_{50}s for the knockout strain compared with the nonmutated parental strain. To demonstrate this magnitude of difference, we studied 20–30 mice of both mutant and wild-type phenotype (20 for halothane and isoflurane, 30 for diethyl ether). Loss of response to a standard tail clamp was used as the anesthetic endpoint. Anesthetic sensitivities were determined for halothane (Halocarbon Laboratories, River Edge, NJ), isoflurane (Baxter Pharmaceutical Products, Liberty Corner, NJ), and diethyl ether (Sigma Chemicals, St. Louis, MO). All animals were between 6 and 10 weeks of age. Equal numbers of both sexes were included in the data after we determined that there were no sex-specific differences to anesthetic sensitivity in these animals. Animal weights were not recorded.

EC_{50}s were determined in nematodes as described in detail previously. Briefly, the response of populations of nematodes was measured at multiple anesthetic concen-

Table 1. EC_{50}s of Wild-type and Mutant Strains of Nematodes and Mice for Three Anesthetics

EC_{50}	N2 (*Ce*)	*unc-1(e580)* (*Ce*)	*unc-1(fc53)* (*Ce*)	129/J (*Mm*)	*stomatin*$^-$ (*Mm*)
Halothane	3.2 (0.1)	3.3 (0.2)	3.3 (0.2)	1.29 (0.09)	1.23 (0.1)
Isoflurane	6.5 (0.2)	6.6 (0.3)	6.7 (0.3)	0.85 (0.06)	0.88 (0.1)
Diethyl ether	5.8 (0.3)	4.8 (0.3)*	4.7 (0.2)*	1.95 (0.08)	1.67 (0.09)*

EC_{50}s (SDs) of wild-type and mutant strains of *Caenorhabditis elegans* (*Ce*) and *Mus musculus* (*Mm*).

* Different from the value for the wild-type strain of the respective species, $P < 0.01$ after a Bonferroni correction for three samples. Note that the only significant differences are for the EC_{50}s in diethyl ether.

trations. The percent of animals immobilized at each dose was compared with the anesthetic concentration to generate dose–response curves using the quantal data. EC_{50}s for nematodes were determined as described by Waud.[24] Anesthetic concentrations necessary for mouse MACs were determined by bracketing as previously described.[22,23] The concentration midway between the highest dose at which animals moved and the lowest dose at which they did not move in response to tail clamping was taken as the MAC for each individual animal. The average of the individual values was used as the MAC for the population.

Statistical Analysis

Dose–response curves for nematodes were constructed as previously described.[5,8] EC_{50}s and SDs were determined by the method of Waud.[21] For each organism, because multiple anesthetics were tested, a Bonferroni correction was applied (for $n = 3$) to test for significance. This decreased the acceptable P value for significance to 0.05/3 or 0.016. For each anesthetic, percentage difference in EC_{50}s from those of wild type were compared for four measurements (wild-type mouse and nematode, mutant mouse and nematode) using analysis of variance. Significance was defined as $P < 0.05$.

Results

The stomatin deletion strain of *C. elegans* (*unc-1(fc53)*) has an uncoordinated motion described as a kinker. The animals are capable of moving well in backward motion; however, in forward motion, the animals often assume "kinked" positions and are unable to move forward effectively. No other locomotion abnormalities have been noted. The stomatin knockout strain of the mouse has no obvious phenotypic abnormalities; in particular, motion seems normal.

Data regarding the EC_{50}s of different strains of *C. elegans* have previously been published.[5,8] We obtained data for two different alleles of *unc-1* animals again in the same time period in which the mouse data were being collected, in a manner previously described.[5] These are the data that are reported in the table and agree with our previous report. As was previously reported, both alleles of *unc-1* caused little to no change in sensitivity to halothane or isoflurane when compared with the wild-type nematode, N2. However, a 15% increase in sensitivity to diethyl ether was noted in both alleles (table 1).

The wild-type mouse strain showed EC_{50}s for halothane and isoflurane similar to those reported by Sonner *et al.*.[22] The EC_{50} for diethyl ether for this strain has not been reported previously. Overt behavioral or anatomical defects in the *stomatin*$^-$ strain of mice were not appreciated by any observers in our laboratory, nor were any defects reported in the previous description of this strain.[17] They displayed an EC_{50} for halothane of 1.23 ± 0.1% compared with 1.29 ± 0.09% for the wild type (table 1). The difference was not significant. The *stomatin*$^-$ strain had an EC_{50} for isoflurane of 0.85 ± 0.06% compared with 0.88 ± 0.1% for the wild type (table 1). The difference was not significant. The *stomatin*$^-$ strain had an EC_{50} for diethyl ether of 1.67 ± 0.09% compared with 1.95 ± 0.08% for the wild type ($n = 30$ for both strains; table 1). The difference was significant at the 0.01 level.

Comparison of differences between mutant and wild-type animals for both mice and nematodes are shown in figure 1. Similar to the nematode, *stomatin*$^-$ mice were approximately 14% more sensitive to diethyl ether than were wild-type animals. There were no differences in sensitivity to halothane or isoflurane between wild-type and *stomatin*$^-$ mice.

Discussion

In a manner exactly analogous to the worm model that prompted this study, knockout of a neuronal stomatin in mice causes the animal to be specifically hypersensitive to the volatile anesthetic diethyl ether. Loss of this gene does not change the sensitivity of the animal to halothane or isoflurane and thus cannot represent a general decrease in nociception or mechanosensation. The ability to correlate a finding in nematodes to the behavior of a mouse in volatile anesthetics is striking and lends strength to the use of *C. elegans* as a model system for the action of anesthetics in mammals. In addition, these findings indicate that immobility in the nematode is predictive of an accepted anesthetic endpoint in the mouse, *i.e.*, response to tail clamp.[22]

Our findings do not indicate that stomatin represents a

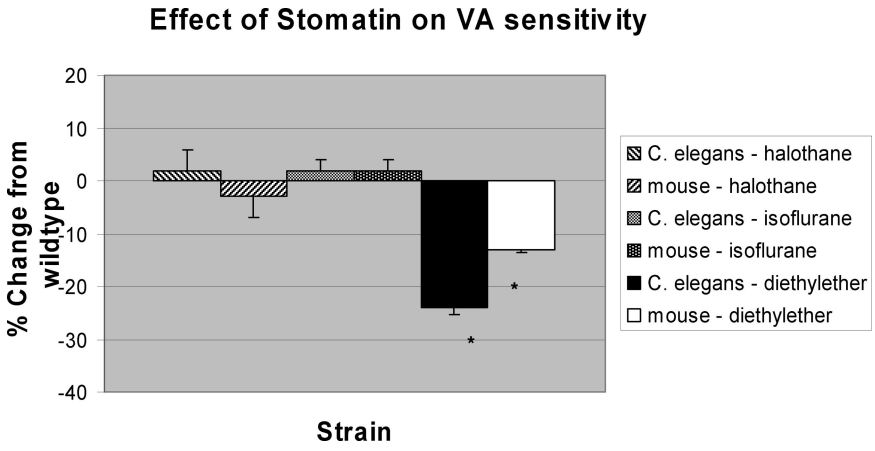

Fig. 1. Percent changes in EC_{50}s compared with the wild-type strain in the mouse and *Caenorhabditis elegans*. The *zero line* refers to the EC_{50} for the wild type, and the *bar graphs* give the percent change of the mutant animals. A negative change indicates a decrease in EC_{50} (an increase in sensitivity). Conversely, a positive change indicates an increase in EC_{50} (a decrease in sensitivity). Note that the pattern of change is similar for mouse and nematode. * Significant difference from the value for the respective wild type animal, $P < 0.05$. Analysis of variance was used to evaluate the differences for multiple measurements. VA = volatile anesthetic.

unique target for volatile anesthetics. In particular, only sensitivity to diethyl ether is affected by a loss of function mutation in the stomatin genes studied. However, in nematodes, other alleles of *unc-1* affect sensitivity to all volatile anesthetics studied.[9] In addition, in the presence of other mutations that alter anesthetic sensitivity, loss of function alleles of *unc-1* affect sensitivity to multiple anesthetics.[8] Therefore, stomatin seems to play a role in a physiologic process affected by volatile anesthetics. What is most important is that mutations in stomatin are capable of altering anesthetic sensitivity across phylogenetic boundaries. This finding lends strength to the use of the nematode as a useful model for anesthetic action in mammals.

Stomatins are integral membrane proteins that are thought to direct trafficking of membrane components and lipid rafts[25,26] as well as to physically interact with ion channels.[12,27,28] *unc-1* encodes a stomatin that is widely expressed in the nervous system of the nematode at all stages of development.[10] The alleles whose anesthetic sensitivities are reported here are null alleles, *i.e.*, little to no functional UNC-1 protein is made in this animal.[10] *unc-1* was the first uncoordinated mutant mapped in the nematode; loss of function alleles confer a striking "kinked" motion in room air.[18] The predicted protein in worms is 87% similar to the protein that is encoded by the mouse gene that we studied (see also Materials and Methods). Stomatins are also widely expressed in mammalian neurons. For example, it has been shown that stomatin is expressed in all neurons within the mouse dorsal root ganglion,[14] in the rat trigeminal neurons, and in whisker follicles.[15]

The precise mechanisms by which stomatin mutations alter anesthetic sensitivity remain unclear. Work in nematodes as well as in other systems reveal an important interaction of stomatins with receptor ion channels known variably as degenerins/epithelial sodium channels (DEG/ENaCs) and acid-sensing ion channels.[9,12,27–29] This superfamily of receptor channels has been implicated in modulating nociception and mechanosensation in peripheral tissues, as well as playing a role in learning and synaptic plasticity in the central nervous system (for a review, see Waud[24]). In nematodes, a stomatin-like protein, MEC-2, has been well characterized in its interaction with ENaCs in the mediation of mechanosensation.[28] (In *C. elegans* terminology, genes affecting mechanosensation are termed *mec* genes; their protein products are termed MECs.) Electrophysiologic studies from the same group showed that the stomatin-like protein MEC-2 strongly affected the function of the MEC-4/MEC-10 ENaCs.

UNC-1 also interacts with a member of the DEG/ENaC superfamily, UNC-8, to control both anesthetic sensitivity and motion in air.[9] UNC-8 is known as a degenerin; mutations in *unc-8* lead to a specific pattern of degeneration in select motorneurons.[29] *unc-8* animals have their own profile of anesthetic response that is allele specific; however, the gain of function alleles have the same specific increase in sensitivity to diethyl ether as do loss of function alleles of *unc-1*. Both UNC-1 and UNC-8 have been shown to localize to lipid raft microdomains in the cell membrane and to coimmunoprecipitate with each other.[27] However, the precise nature of the interaction of these proteins remains unknown. We showed that a subset of the DEG/ENaC class of channels is sensitive to volatile anesthetics and that different mutations affecting these channels are therefore capable of causing specific changes in sensitivity.[9] Alternatively, these channels may affect other downstream molecules, which are themselves anesthetic targets. The latter model is more fitting with the involvement of *unc-1* and *unc-8* as suppressors of multiple other mutations altering anesthetic sensitivity.[8,9]

Stomatins are known to interact with DEG/ENaCs in mammals and mammalian cell lines. Stomatin colocalizes with α-, β-, and γ-ENaC subunits in the trigeminal ganglion and in mechanoreceptive nerve terminals in whisker follicles of the rat.[15] The interaction of the two proteins is postulated to be important in transducing mammalian mechanosensation. Stomatins also interact with acid-sensing ion channels, channels important for both mechanoreception as well as nociception. Stom-

atins colocalize and coimmunoprecipitate with acid sensing ion channels when heterologously expressed in COS-7 cells. Stomatin altered the function of acid-sensing ion channel gating when expressed in Chinese hamster ovary cells.[12]

The similarity of the interaction of mammalian stomatin with DEG/ENaCs compared with that of nematode proteins MEC-2/MEC-4/MEC-10 has strengthened the conclusion that stomatins are important proteins that mediate mechanosensation in mammals. However, until this study, no one has investigated whether stomatin is important in controlling anesthetic sensitivity in the mouse. This study shows a near-identical behavior in mouse and nematode knockouts of neuronal stomatin. Whether this occurs through a direct interaction of anesthetics with stomatin, through the effects of stomatin on DEG/ENaCs, or through an effect of volatile anesthetics on a protein complex that includes these molecules is not known. It is important to be cognizant of the differences between nematodes and mammals. Although deficiencies in stomatin lead to similar changes in anesthetic sensitivities in these two animals, it remains possible that the molecular causes of these changes may differ greatly. It is possible that stomatin interacts with widely different targets in the two organisms to cause these changes, for example. It is also important to note that compensatory changes are often seen in mouse knockout models, although seen less often in nematodes. As a result, changes in the expression of other genes may actually underlie the alterations in anesthetic responses. However, the results lend plausibility to the use of immobility in *C. elegans* as a model for MAC in mammals and indicate that findings in invertebrate model systems are likely to be of use in understanding physiologic mechanisms of anesthetic action in mammals.

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References

1. Baumeister R, Ge L: The worm in us: *Caenorhabditis elegans* as a model of human disease. Trends Biotechnol 2002; 20:147–8

2. Nass R, Blakely RD: The *Caenorhabditis elegans* dopaminergic system: Opportunities for insights into dopamine transport and neurodegeneration. Annu Rev Pharmacol Toxicol 2003; 43:521–44

3. Campagna JA, Miller KW, Forman SA: Mechanisms of actions of inhaled anesthetics. N Engl J Med 2003; 348:2110–24

4. Eger EI II, Fisher DM, Dilger JP, Sonner JM, Evers A, Franks NP, Harris RA, Kendig JJ, Lieb WR, Yamakura T: Relevant concentrations of inhaled anesthetics for *in vitro* studies of anesthetic mechanisms. ANESTHESIOLOGY 2001; 94:915–21

5. Morgan PG, Sedensky MM, Meneely PM, Cascorbi HF: The effect of two genes on anesthetic response in the nematode *Caenorhabditis elegans*. ANESTHESIOLOGY 1988; 69:246–51

6. Kayser EB, Morgan PG, Sedensky MM: GAS-1: A mitochondrial protein controls sensitivity to volatile anesthetics in the nematode *Caenorhabditis elegans*. ANESTHESIOLOGY 1999; 90:545–54

7. Morgan PG, Hoppel CL, Sedensky MM: Mitochondrial defects and anesthetic sensitivity. ANESTHESIOLOGY 2002; 96:1268–70

8. Morgan PG, Sedensky M, Meneely PM: Multiple sites of action of volatile anesthetics in *Caenorhabditis elegans*. Proc Natl Acad Sci U S A 1990; 87:2965–9

9. Rajaram S, Spangler TL, Sedensky MM, Morgan PG: A stomatin and a degenerin interact to control anesthetic sensitivity in *Caenorhabditis elegans*. Genetics 1999; 153:1673–82

10. Rajaram S, Sedensky MM, Morgan PG: Unc-1: A stomatin homologue controls sensitivity to volatile anesthetics in *Caenorhabditis elegans*. Proc Natl Acad Sci U S A 1998; 95:8761–6

11. Sedensky MM, Siefker JM, Morgan PG: Model organisms: New insights into ion channel and transporter function. Stomatin homologues interact in *Caenorhabditis elegans*. Am J Physiol Cell Physiol 2001; 280:C1340–8

12. Price MP, Thompson RJ, Eshcol JO, Wemmie JA, Benson CJ: Stomatin modulates gating of acid-sensing ion channels. J Biol Chem 2004; 279:53886–91

13. Goldstein BJ, Kulaga HM, Reed RR: Cloning and characterization of SLP3: A novel member of the stomatin family expressed by olfactory receptor neurons. J Assoc Res Otolaryngol 2003; 4:74–82

14. Mannsfeldt AG, Carroll P, Stucky CL, Lewin GR: Stomatin, a MEC-2 like protein, is expressed by mammalian sensory neurons. Mol Cell Neurosci 1999; 13:391–404

15. Fricke B, Lints R, Stewart G, Drummond H, Dodt G, Driscoll M, von During M: Epithelial Na+ channels and stomatin are expressed in rat trigeminal mechanosensory neurons. Cell Tissue Res 2000; 299:327–34

16. Fricke B, Jarvis HG, Reid CD, Aguilar-Martinez P, Robert A, Quittet P, Chetty M, Pizzey A, Cynober T, Lande WF, Mentzer WC, During M, Winter S, Delaunay J, Stewart GW: Four new cases of stomatin-deficient hereditary stomatocytosis syndrome: association of the stomatin-deficient cryohydrocytosis variant with neurological dysfunction. Br J Haematol 2004; 125:796–803

17. Zhu Y, Paszty C, Turetsky T, Tsai S, Kuypers FA, Lee G, Cooper P, Gallagher PG, Stevens ME, Rubin E, Mohandas N, Mentzer WC: Stomatocytosis is absent in "stomatin"-deficient murine red blood cells. Blood 1999; 93:2404–10

18. Brenner S: The genetics of *Caenorhabditis elegans*. Genetics 1974; 77: 71–94

19. Herman RK: Genetics of *C. elegans*, The Nematode *Caenorhabditis elegans*. Edited by Wood WB. Cold Spring Harbor, New York, Cold Spring Harbor Laboratory Press, 1988, pp 22–33

20. Horvitz HR, Brenner S, Hodgkin J, Herman RK: A uniform genetic nomenclature for the nematode *Caenorhabditis elegans*. Mol Gen Genet 1979; 175: 129–33

21. Rajaram S, Sedensky MM, Morgan PG: The sequence and associated null phenotype of a *C. elegans* neurocalcin-like gene. Genesis 2000; 26:234–9

22. Sonner JM, Gong D, Li J, Eger EI II, Laster MJ: Mouse strain modestly influences minimum alveolar anesthetic concentration and convulsivity of inhaled compounds. Anesth Analg 1999; 89:1030–4

23. Quasha AL, Eger EI II, Tinker JH: Determination and application of MAC. ANESTHESIOLOGY 1980; 53:315–34

24. Waud DR: On biological assays involving quantal responses. J Pharmacol Exp Ther 1972; 183: 577–607

25. Morrow IC, Parton RG: Flotillins and the PHB domain protein family: rafts, worms and anaesthetics. Traffic 2005; 6:725–40

26. Mairhofer M, Steiner M, Mosgoeller W, Prohaska R, Salzer U: Stomatin is a major lipid-raft component of platelet alpha granules. Blood 2002; 100:897–904

27. Sedensky MM, Siefker JM, Koh JY, Miller DM III, Morgan PG: A stomatin and a degenerin interact in lipid rafts of the nervous system of *Caenorhabditis elegans*. Am J Physiol Cell Physiol 2004; 287:C468–74

28. Goodman MB, Ernstrom GG, Chelur DS, O'Hagan R, Yao CA, Chalfie M: MEC-2 regulates *C. elegans* DEG/EnaC channels needed for mechanosensation. Nature 2002; 415:1039–42

29. Tavernarakis N, Shreffler W, Wang S, Driscoll M: unc-8, a DEG/EnaC family member, encodes a subunit of a candidate mechanically gated channel that modulates *C. elegans* locomotion. Neuron 1997; 18:107–19