Published in final edited form as:

J Phys Chem B. 2013 December 19; 117(50): 16141–16147. doi:10.1021/jp411261g.

Xenon and Other Volatile Anesthetics Change Domain Structure in Model Lipid Raft Membranes

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Abstract

Inhalation anesthetics have been in clinical use for over 160 years, but the molecular mechanisms of action continue to be investigated. Direct interactions with ion channels received much attention after it was found that anesthetics do not change the structure of homogeneous model membranes. However, it was recently found that halothane, a prototypical anesthetic, changes domain structure of a binary lipid membrane. The noble gas xenon is an excellent anesthetic and provides a pivotal test of the generality of this finding, extended to ternary lipid raft mixtures. We report that xenon and conventional anesthetics change the domain equilibrium in two canonical ternary lipid raft mixtures. These findings demonstrate a membrane-mediated mechanism whereby inhalation anesthetics can affect the lipid environment of trans-membrane proteins.

Keywords

membrane; lipid raft; membrane domain; anesthesia; xenon; diffraction

Introduction

The noble gas, xenon, is an excellent anesthetic ¹. It has very little chemical reactivity and a minimum alveolar concentration (MAC) of 0.6 to 0.7 atmospheres. Interest has developed in its use as general anesthetic and neuroprotectant ², just as conventional anesthetics have been studied as neuro- and cardioprotectants. Xenon inhibits NMDA neurotransmitter receptors by competitive binding to the glycine binding site ³. There is also evidence for non-competitive inhibition of NMDA receptors due to binding at another site, as yet unidentified⁴. In addition, two pore domain potassium channels TREK-1 ⁵ and ATP-sensitive potassium channels are activated by xenon, causing hyperpolarization of cell membranes ⁶. While these interactions may be major contributors to xenon's action as general anesthetic and neuroprotectant, molecular effects of xenon and other inhalation anesthetics need careful investigation to aid clinical evaluations⁷, including side effects of anesthesia ^{6,8}. Conventional inhalation anesthetics such as isoflurane interact with neurotransmitter receptors very differently than xenon ⁹. This suggests that there are several different mechanisms of inhalation anesthetic action. Mechanisms not involving direct

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Supporting Information: Additional data on response of lipid mixtures to anesthetics is available free of charge via the internet at <http://pubs.acs.org>.

interactions with proteins are also possible. It was previously demonstrated that halothane, a prototypical anesthetic, changes the domain structure of binary lipid membranes¹⁰. Xenon provides a test of the generality of this finding, extending it to volatile anesthetics beyond halogenated hydrocarbons. Current pictures of plasma membranes involve complex lipid mixtures, with laterally separated domains that result from lipid de-mixing¹¹ and specific membrane proteins clustered in cholesterol- and sphingomyelin-rich domains called “lipid rafts”¹², such as in caveolae. X-ray and neutron diffraction have shown that halothane, but not the non-immobilizer F6 (dichlorohexafluorocyclobutane), produces a redistribution of lipid from the ordered to the disordered phase¹⁰ in a model binary lipid mixture of dipalmitoylphosphatidylcholine (DPPC) and dilauroylphosphatidylcholine (DLPC) 1:1, a well characterized system that forms distinct DPPC-rich ordered and DLPC-rich fluid phases¹³.

Ternary mixtures of phospholipids and cholesterol are thought to be more representative of cell membranes with lipid rafts, so the binary mixture studies were extended to these mixtures. Model lipid raft mixtures are classified as Type I (solid or liquid-ordered (L_o) and liquid disordered (L_d) phases with no microscopically visible phase separation) and Type II (with visible phase separation)¹⁴. We chose two well-studied Type II mixtures with similar mixing transition temperatures (Supporting Information): dioleoylphosphatidylcholine (DOPC)/DPPC/cholesterol 1:1/0.2 (equi-molar DOPC and DPPC with cholesterol making up 20% of total lipid) and DOPC/sphingomyelin(SM)/cholesterol 1:1/0.2¹⁴. Effects of xenon and conventional inhalational anesthetics were studied using lamellar neutron and X-ray diffraction.

Experimental Methods

Materials

Lipids were obtained from Avanti Polar Lipids, chloroform, halothane and F6 from Sigma, isoflurane from Baxter, xenon 99.995% purity from Matheson and Linde, nitrous oxide from Airgas, and helium from Roberts. Highly oriented multi-lamellar stacks of lipid bilayers at 1 to 2 mg/cm² were formed on thin microscope cover glass substrates, as previously described¹⁰, by slow evaporation of solvent from solutions in ethanol or 80% ethanol/20% water at 37 to 40°C in air, followed by 15 minutes in vacuum.

Neutron Methods

Neutron diffraction data were obtained with the Advanced Neutron Diffractometer/Reflectometer¹⁵ with momentum transfer perpendicular to the bilayer planes. Deuterated d-62 DPPC and d-31 palmitoyl sphingomyelin were used to increase contrast for neutrons scattered by the L_o phases of these mixtures and give larger changes with domain mixing. Discrete diffraction peaks produced by the L_o and L_d phases result from the different d-spacings for these phases, with the L_d phase having shorter spacing. Although we readily obtained 4 orders of neutron diffraction for each phase, intensities for third and fourth orders were much smaller than those of the first two, so for rapidly measuring effects of xenon, only the first two orders were used. We constructed a sealed chamber of aluminum, pressure tested to 8 atmospheres, to enclose the lipid samples. Chamber temperature was controlled by circulating a thermo-controlled liquid through its base, and monitored with sensors in the bath and attached to different points on the chamber. Humidity was maintained at 98% with saturated salt solution¹⁶. Gases were slowly introduced into the chamber through a bubbler filled with distilled water to aid constant hydration of the lipid multi-layers. Particular care was taken when changing pressure in the chamber to avoid rapid pressure changes and maintain constant temperature and humidity.

The glass slides with deposited multilayers were rotated in the incident cold-neutron beam (wavelength $\lambda = 5.0 \text{ \AA}$, $\Delta\lambda/\lambda = .01$) through angles Θ relative to the incident beam¹⁵. Diffracted neutrons were detected at angles 2Θ (referred to as Θ – 2Θ scans). Thus, the neutron momentum transfer ($Q_z = 4\pi\sin\Theta/\lambda$) normal to the bilayer plane probes the structure of the membrane along the bilayer normal. A high efficiency pencil detector registered two series of distinct diffraction peaks for each sample.

X-ray methods

X-ray measurements used a Rigaku Ultima III diffractometer. An X-ray chamber with a Peltier-controlled base (CP-031 TE Technology) was constructed for temperature control. Samples were supported by the Peltier base and in direct contact with it. Temperature was controlled to within 0.1° C . Kapton windows provided X-ray access. The aluminum top plate of the chamber had ports for introducing anesthetic/hexadecane solutions and for sampling gas concentrations, as well as a miniature fan to promote gas mixing in the chamber. The fan was baffled to prevent direct flow onto the sample. 98% relative humidity was maintained by saturated solutions of K_2SO_4 . Temperature and humidity were monitored with sensors inside the chamber. Stable vapor concentrations of anesthetics were obtained by adding solutions of anesthetics with hexadecane. Such solutions are very close to ideal, and vapor pressures follow Raoult's law, so the solution provides a reservoir of anesthetic at essentially constant chemical potential¹⁷. Concentrations of anesthetic were measured by withdrawing samples of gas into 5 ml syringes and analyzing these on an Agilent 6850 gas chromatograph. Electron density profiles were computed using direct Fourier reconstruction¹⁸. At least two experiments were performed for each lipid mixture and each anesthetic. For xenon experiments we used a miniature chamber constructed from plastic cuvettes with a 1 cm optical path. Humidity was controlled as above.

Electron density profiles

Electron density profiles were calculated using four orders of diffraction for both the DPPC and SM mixtures to determine whether the change in phase distributions induced by anesthetics demonstrated by the change in ratio of the magnitudes of the first order diffraction peaks accurately reflected the change in total mass of each phase in the mixture. For both mixtures, the change in ratio of the heights of the 1st order L_d and L_o peaks was very close to twice the change in ratio of the amplitudes of the calculated electron density profiles. The structure factors vary as the square root of intensity, so for small changes, as intensity divided by 2. Thus, ratios of 1st order peaks dominate changes of profiles, as expected since the first order intensities are two orders of magnitude larger than the higher order peaks and we observed that ratios of higher order peaks changed in the same direction with anesthetics. However, we also note that mass changes in these samples probably change in complex ways: disappearance of mosaic blocks, changes in size of mosaic blocks, or changes in the number of layers in mosaic blocks. The first two changes should scale with intensities, while the last would scale with structure factor. In addition, lipids may move into and out of membrane regions connecting domains of different d-spacing. These connecting regions do not contribute to lamellar diffraction in Θ – 2Θ scans.

Comparisons between Neutron and X-ray data

Connecting domains complicate the quantitative evaluation of neutron and X-ray diffraction data since they do not contribute to the main lamellar diffraction and are variable between samples. Another complexity arises from the change in bilayer structure factors caused by using deuterium in the lipid chains. Deuterium provides good signal for neutron diffraction but causes substantial changes in structure factors as lipids mix, which is not the case with X-rays. To obtain the scale factor between neutron and X-ray data we compared the change

in peak areas observed with the neutron beam at 1.5 mol % halothane on the deuterated DPPC/DLPC sample (46%) with the best linear fit to the X-ray data for DPPC/DLPC and halothane (26% at 1.5 mol%). This scale factor is only approximate, as it does not account for the difference in lipid composition between the d31-palmitoyl sphingomyelin used in the neutron beam and the porcine sphingomyelin used in the X-ray experiments. The complex mixture of lipids in the porcine sphingomyelin may also account for the somewhat lower sensitivity of these samples to anesthetics.

Minimum Alveolar Concentrations

Data for humans at 37° C are available for xenon ^{1, 2} halothane, isoflurane and nitrous oxide ^{8, 19}. The lower temperatures used in this study would increase the solubility of xenon in the lipid bilayers only very modestly, about 15% ²⁰. Chloroform was abandoned for clinical use in the early part of the 20th century due to reports of liver toxicity. We use the average of the concentration necessary to inhibit the righting response and the MAC in mice ²¹ as an estimate of the MAC for chloroform in humans. For both halothane and isoflurane this average is a very good estimate of the MAC in humans.

Results and Discussion

Figure 1 illustrates the effect of xenon on the first order peaks of DPPC/DOPC/cholesterol and DOPC/SM/cholesterol samples, at 28° C and 27° C, respectively, and 98% humidity. In air or helium, diffraction from the L_o phase predominates. The chamber containing the DPPC sample was flushed with xenon and then pressurized with xenon to twice and then four times atmospheric pressure equivalent to 3.2 and 6.4 times the minimum alveolar concentration (MAC). Xenon produced a marked increase of the L_d phase in both the first and second order peak intensities. At 3.2 MAC the ratio of L_o to L_d intensities decreased by 21%, and at 6.4 MAC by 35%. The sphingomyelin sample demonstrates a 39 % change in the ratio of L_o to L_d intensities in response to xenon at 4.6 MAC. The decrease in the L_o phase only partially accounts for the increase in the L_d phase. The complex structure of the aligned multi-layer stacks, with connecting elements between L_o and L_d domains not oriented to the bilayer normal ^{22,23}, accounts for this. Reducing the pressure of xenon to atmospheric pressure resulted in a prompt reversal of the intensity ratio giving an increase in L_o and decrease in L_d with the ratio for 1 atmosphere xenon for both mixtures remaining about 14% below that observed in air.

Changes in the L_o/L_d intensity ratio observed by neutron diffraction for both the DOPC/DPPC/cholesterol mixture and the SPM/DOPC/cholesterol mixture as a function of anesthetic concentration (both xenon and nitrous oxide) are shown in Figure 2. Each mixture was studied at a temperature within its mixing transition zone (Supporting Information). Concentrations of anesthetic normalized by minimum alveolar concentration (MAC) produced comparable changes in L_o/L_d intensity ratios. A sample of DOPC/DPPC/cholesterol was studied at two different temperatures (27° C and 31° C). Xenon at 3.1 MAC produced changes in the L_o/L_d ratios of 19% and 22% respectively. Thus, effects of anesthetics on changes in L_o/L_d phase distribution do not depend greatly upon temperature as long as the sample is within its mixing transition zone. While there is some variation between samples, the overall correlation (adjusted $r^2 = 0.89$) between physiological dose and change in L_o/L_d intensity ratios is good. Individual samples demonstrated a largely linear response to increasing pressure of anesthetics, as illustrated in Figure 3 by the response of a sample of d-DPPC/DOPC/cholesterol to increasing pressures of xenon and nitrous oxide. In this sample, the responses to xenon and nitrous oxide as a function of pressure (rather than MAC) are nearly the same.

To establish that the effects observed were not due to pressure effects on the membranes, the DOPC/DPPC/cholesterol sample was studied in helium at different pressures. Helium at 5 atmospheres produced no discernible change in the intensities of diffraction peaks. Pressure reversal of general anesthesia generally requires about 100 atmospheres pressure.

Effects of more conventional anesthetics on both lipid raft mixtures were studied using X-ray diffraction and can be compared with the neutron results for xenon and nitrous oxide. Annealing and equilibration of samples in the X-ray chamber was important for obtaining stable and reversible data, consistent with the kinetics of domain evolution in stacked lipid multi-layers (17). Diffraction patterns indicating two distinct lamellar phases for mixtures of DOPC/DPPC/cholesterol (1:1/0.2) and DOPC/SM (porcine)/cholesterol (1:1/0.2) were obtained, as reported previously^{10, 22, 23}, consistent with the formation of aligned, multi-lamellar membrane stacks. As found with the binary DPPC/DLPC mixture¹⁰ and with xenon in the studies described above, administration of anesthetics such as isoflurane and halothane at physiologically relevant concentrations produced shifts in the distribution of disordered to ordered liquid phases in the membranes, as indicated by changes in magnitudes of the diffraction peaks. Concentrations of anesthetics in the bilayers did not approach levels necessary for the electron-rich halogens to change X-ray structure factors for the bilayers²⁴. The same shift to favor the disordered phase was observed for both DOPC/DPPC/cholesterol and DOPC/SM/cholesterol in the presence of isoflurane, halothane and chloroform (Table 1), as well as hexane (Supporting Information). The magnitudes of changes in the lipid phase distribution are not equal for the neutron and X-ray diffraction because of the different scattering length densities and changes of deuterium content in L_o and L_d for neutron diffraction (Experimental Methods). Extrapolating from the comparison of neutron and X-ray measurements of the anesthetic induced shifts in the DPPC/DLPC mixture, we estimate that the 39% change in L_o/L_d neutron intensity ratio in DOPC/SM/cholesterol induced by xenon at 4.6 MAC corresponds to a 22% change measured by X-rays, and is comparable to the changes observed with halothane and isoflurane.

Inhalation anesthetics thus produce changes in L_o/L_d phase mixing somewhat comparable to an increase in temperature. Unlike temperature changes, anesthetics do not produce significant changes in bilayer structure and d-spacing. Changes in d-spacing caused by anesthetics were sub-Ångström and variable. Chloroform and isoflurane produced changes in d-spacing less than 0.15 Ångströms, while halothane at the highest doses used produced a decrease of 0.3 Ångströms (Supporting Information). The response of the DPPC/DOPC/cholesterol mixture to increasing temperature is illustrated in Figure 4, and demonstrates the well established decrease in membrane thickness corresponding to an increase in area per lipid²⁵. In contrast to anesthetics, administration of the non-immobilizer, F6, at equivalent concentrations²⁶ produced no change in the phase distribution of the DOPC/SM/cholesterol mixture and only a very small ($6\pm 2\%$) change in that of the DOPC/DPPC/cholesterol mixture.

To further investigate membrane effects of xenon, we studied effects of xenon on pure DOPC membranes by X-ray diffraction, using a chamber with 1 cm path length to minimize X-ray absorption. Figure 5 shows that xenon at atmospheric pressure produced a substantial decrease in diffraction, but when scaled to account for absorption, the electron density plot for DOPC with xenon was identical with that obtained with air at the same hydration. This finding is consistent with simulations of membranes with xenon at atmospheric pressure²⁷. Thus, xenon at atmospheric pressure appears to have no effect on the X-ray structure of a single-phase phospholipid bilayer, just as other anesthetics at similar MAC had no effect on homogeneous phospholipid/cholesterol bilayers²⁸. At higher pressures of xenon, this “heavy atom” for X-rays has been used to map detergent regions as well as binding sites in crystals of membrane proteins²⁹, and simulations suggest that xenon will distribute towards

the center of the membrane as pressures increase^{27, 30}. Partitioning of xenon into hydrocarbon regions, which follows the Meyer-Overton rule, is largely due to its size and polarizability, which are very similar to methane.

Thus, neither xenon nor conventional inhalational anesthetics produce the expansion in area per lipid seen with increasing temperature. The increases in the disordered phase produced by anesthetics could produce increases in lateral pressure profiles as first proposed by Gruner and Shyamsunder³¹ and then refined by Cantor³². As these and subsequent authors¹⁰ noted, such lateral pressure profile changes are of appropriate magnitude to cause concomitant changes in trans-membrane protein conformations.

The bilayers studied in these experiments were not derived from living cells, but rather represent models of membranes containing lipid rafts. Lipid rafts are not optically visible in mammalian cells¹², so the domain areas in cells must be orders of magnitude smaller than those of the membranes studied here. The ratio of surface area to volume of such domains is much higher than those of the domains in the present study, and thus, these domains should be far more sensitive to the effects of anesthetics. In addition, the domains studied here are three-dimensional. Even so, the levels of anesthetics required to demonstrate effects in the membranes studied here are comparable to clinical concentrations. It is desirable to study the effects of anesthetics on bilayers with nano-domains (Type 1 lipid raft mixtures), but vertical stacking of nano-domains has not yet been demonstrated, so diffraction methods have not been applied to them.

Do small changes in d-spacing contribute to changes in the intensities of diffraction peaks? In this case we would expect to see amplitudes of first order and second order diffraction peaks change oppositely. As noted for the data in figure 1, first and second order diffraction peaks for the ordered phase, both decreased while those of the disordered phase increased. Moreover, decreases in the ratios of intensity for ordered phase to disordered phase were observed at all orders of diffraction (see Supporting Information for an example with four diffraction orders). These findings and the absence of significant change in d-spacing are inconsistent with d-spacing changes causing major intensity changes. Nyquist-Shannon sampling theory demonstrates this, both qualitatively and quantitatively. Applying the sampling theorem to the xenon data, the maximal 1% increase in d-spacing of the disordered phase in Figure 1, as an increase in hydrocarbon thickness, predicts a 2% increase in first order intensity, from the slope of the reconstructed transform at the first order, and thus a 2% decrease in L_o/L_d with L_o fixed, whereas we observe a 35% decrease in L_o/L_d (Figure 3). Nyquist-Shannon theory also predicts that the 2nd order changes would be in the opposite direction, contrary to what we observe. If the unit cell change is an increase across the hydration layer, the predicted 1st and 2nd order changes are reversed. These features are thus qualitatively and quantitatively incompatible with our results.

As noted above, lipid membranes that connect domains of different d-spacing would not be oriented the same as the domains and therefore would not contribute to the lamellar diffraction. The concern then arises that the changes in observed peak amplitudes reflect movement of lipid in and out of this reservoir into domains rather than true mixing of lipids from different domains. (See figure 5 and text in Mills, et al.²² for a thoughtful discussion on this issue.) We believe this is unlikely for several reasons. First, we addressed this issue in detail in our previous study of the d62-DPPC/DLPC mixture using neutron diffraction along the axis parallel to the membrane¹⁰ (in-plane diffraction). This technique capitalizes on the strong scattering by deuterated DPPC chains as compared to the very weak scattering by hydrogen in the DLPC chains. In-plane diffraction intensity therefore depends on deuterated chain correlations, but is completely independent of vertical stacking. Both increasing temperature and introduction of anesthetic decrease the amplitude of the chain

diffraction signal. This can only occur with mixing of the deuterated with the non-deuterated lipids. Secondly, some samples demonstrate a nearly one-to-one relationship between the decrease in ordered phase and increase in disordered phase (figure 1a) and we observe consistent phase mixing changes induced by anesthetics in samples with at least a four-fold range of thickness. Finally, it is difficult to postulate a mechanism that would relieve stacking frustration only for the disordered domains and not for ordered domains.

Conclusions

These neutron and X-ray data demonstrate that a variety of volatile anesthetics all change the phase distribution of lipid raft mixtures to favor the disordered phase. Recent simulations^{33, 34} of anesthetics in model membranes reached similar conclusions. Strikingly, the differential results we obtain with F6, as opposed to the halogenated anesthetics, resemble those found in simulations of chloroform vs. carbon tetrachloride, which is another non-anesthetic³⁵. This similarity should occur given the parallels between experimental data¹⁰ and simulation³⁵ for the preference of anesthetics, as opposed to non-anesthetics, to partition into the disordered phase.

Could these shifts in lipid phase distribution, demonstrated in model membranes, contribute to the “non-specific mechanisms”³⁶ by which volatile anesthetics exert their effects? Shifts in L_o/L_d mixing can produce substantial mechanical^{10, 31, 32} effects on raft containing membrane domains and thus on a variety of cellular processes, including caveolae signaling³⁷. The family of voltage-gated potassium channels appears to be very sensitive to mechanical properties of the membranes in which they are located^{38, 39}. The phenomenon of pressure reversal of anesthesia is also consistent with this picture, since pressure favors the ordered phase⁴⁰. Anesthetics produce a wide array of side effects. For example, exposure to volatile anesthetics during development induces apoptosis of neurons in primate brains⁴¹ and behavioral deficits in *C. elegans*⁴². Mechanisms underlying these and other side effects of anesthetics are yet to be determined and are not necessarily similar to those causing anesthesia. Thus, changes in lipid phase distribution may contribute to anesthesia and side effects of anesthesia. Elucidation of the precise molecular mechanisms⁷ will require detailed study of the effects of anesthetics, direct and indirect, on membrane proteins. This is still a technically difficult challenge.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

Acknowledgments

We thank Drs. Sergey Bezrukov and Hirsh Nanda for their comments, Dr. David Sandstrom for the use of his gas chromatograph, and Dr. Jason Simmons for his assistance with the X-ray diffractometer. DW was supported by US National Institute of Health Grant GM86685 (to Stephen H. White). The Center for Neutron Research provided facilities for neutron and X-ray diffraction. The identification of any commercial product or trade name does not imply any endorsement or recommendation by the National Institute of Standards and Technology.

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Figure 1.

A. Neutron Diffraction. First order diffraction peaks for a multi-layered sample of deuterated d-62 DPPC/DOPC 1:1 with 20% cholesterol. The first peak is from the L_o phase, the second is from the L_d phase. Black trace is for helium at atmospheric pressure, red trace with is for xenon at 2 times atmospheric pressure (3.2 MAC), blue trace is for xenon at 4 times atmospheric pressure (6.4 MAC). Experiments performed at 28° C and 98% relative humidity. Traces are Gaussian fits to data. $Q = 4\pi\sin\Theta/\lambda$ is the neutron momentum transfer. Bars indicate standard errors (1 atmosphere = 101 kPa).

B. Neutron Diffraction. First order diffraction peaks for a multi-layered sample of deuterated d-31 palmitoyl sphingomyelin/DOPC 1:1 with 20% cholesterol. The first peak is from the L_o phase, the second is from the L_d phase. Black trace is for air, red trace is for xenon at 3 times atmospheric pressure (4.6 MAC). Experiments performed at 27° C and 98% relative humidity. Traces are Gaussian fits to data. Bars indicate standard errors (1 atmosphere = 101 kPa).

Figure 2.

Change in ratio first order peak areas vs. MAC. Solid squares are for SPM/DOPC/cholesterol mixture with xenon, solid triangles are for SPM/DOPC/cholesterol mixture with nitrous oxide, open squares are for DPPC/DOPC/cholesterol mixture with xenon, and open triangles for DPPC/DOPC/cholesterol mixture with nitrous oxide. MAC used for xenon is 0.63 atmospheres (4), nitrous oxide 1.04 atmospheres (10). Dashed line represents least squares linear fit, adjusted $r^2 = 0.89$. Bars represent standard errors.

Figure 3.

A. Neutron Diffraction Changes with Xenon. Graph plots the ratio of first order diffraction peak areas for the L_0 and L_d phases during the course of an experiment on a sample of d62-DPPC/DOPC/cholesterol. First set of points was obtained in 100% helium at atmospheric pressure, $T = 28^\circ \text{C}$, $\text{RH} = 98\%$. Sets of points following are the ratios with xenon in the chamber at atmospheric pressure, twice, three, and four times atmospheric pressure, then again at one atmosphere. The last set was obtained at atmospheric pressure after flushing the chamber with helium. (1 atmosphere = 101 kPa).

B. Neutron Diffraction Changes with Nitrous Oxide. Graph plots the ratio of first order diffraction peak areas for the L_0 and L_d phases during the course of an experiment on a sample of d-DPPC/DOPC/cholesterol. First set of points was obtained in air at atmospheric pressure. Sets of points following are the ratios with nitrous oxide in the chamber at atmospheric pressure, twice, and four times atmospheric pressure, then again at one atmosphere. The last set was obtained at atmospheric pressure after flushing the chamber with helium. $T = 28^\circ \text{C}$, $\text{RH} = 98\%$.

Figure 4.

D-spacing changes for the L_o and L_d phases of the DOPC/DPPC/cholesterol mixture, as a function of temperature. The L_o phase demonstrates the usual linear relationship between membrane thickness and temperature (coefficient of thermal expansion), while the L_d phase demonstrates a non-linear effect due to the mixing of DPPC and cholesterol into this phase.

Figure 5.

Effect of xenon on DOPC. Electron density plots derived by Fourier reconstruction from X-ray diffraction by DOPC multi-layers. Experiments performed at 27°C and 98% relative humidity. Solid black trace is for air, dashed blue trace is for xenon at atmospheric pressure, dotted red trace (overlaps black trace) is for xenon at atmospheric pressure with diffraction data scaled for X-ray absorption by xenon.

Table 1

X-ray diffraction data for lipid raft mixtures in the presence of halogenated anesthetics. % $\Delta 1H$ indicates the percentage change in the ratio of first order diffraction peaks L_o/L_d between air and anesthetic. %MAC indicates the mean alveolar concentration used as a percentage of the minimum alveolar concentration necessary for humans (Methods). At least two experiments on separate samples were performed for each anesthetic. Mean values are tabulated with standard errors.

Mixture	Anesthetic	% $\Delta 1H$	%MAC
DOPC:DPPC/cholesterol 1:1/0.2			
	Halothane	31 ± 3	270 ± 60
	Isoflurane	17 ± 2	121 ± 22
	Chloroform	22 ± 2	167 ± 17
DOPC:SM/cholesterol 1:1/0.2			
	Halothane	21 ± 5	540 ± 20
	Isoflurane	20 ± 3	300 ± 60
	Chloroform	25 ± 8	262 ± 26