

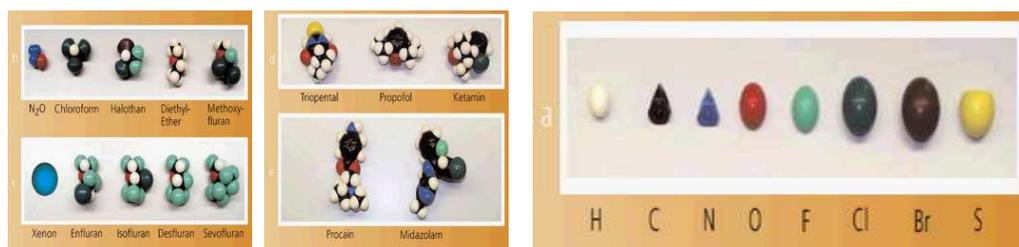
# Function of Nerves - Action of Anesthetics

By

KAARE GRÆSBØLL

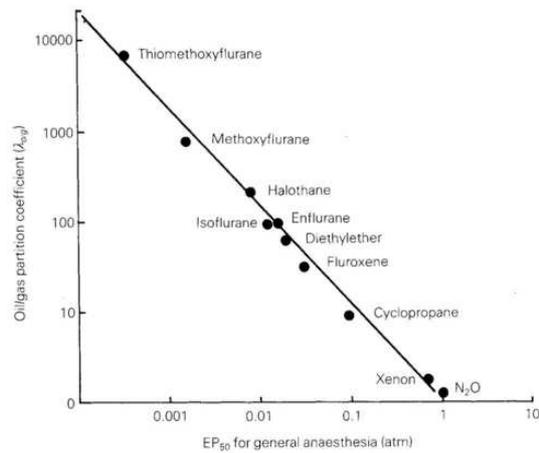
*Kaare Græsbøll is a master student in the Membrane Biophysics Group at the Niels Bohr Institute. Email: karreg@fys.ku.dk*

*If you are having your car fixed, you expect the mechanic to know how your car functions in order to repair it. If you go through surgery, you might expect your surgeon to know how your body functions and the anesthesiologist to know how the anesthesia influence your nervous system. The latter is not the case! While the auto mechanic knows every bolt and the surgeon every vein, the anesthesiologist cannot give you a straight answer. Why? - Science simply has not provided a clear answer. In the Membrane Biophysics Group (MBG) at NBI we are working on a new radically different model of nerves and anesthetic action. But first a short introduction to the field:*



From Anästhesie in Zürich: 100 Jahre Entwicklung 1901-2001: Die MeyerOvertonRegel: Was ist geblieben? Bernd W. Urban

Above there is a small collection of molecules. They vary from single atom to complex molecules, but despite their obvious differences they all act as anesthetics. The common properties of these drugs were discovered more than a hundred years ago by C.E. Overton:

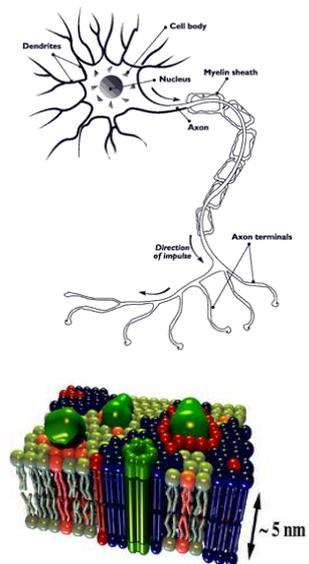


C. E. Overton, Studies of Narcosis (Chapman & Hall, 1991).

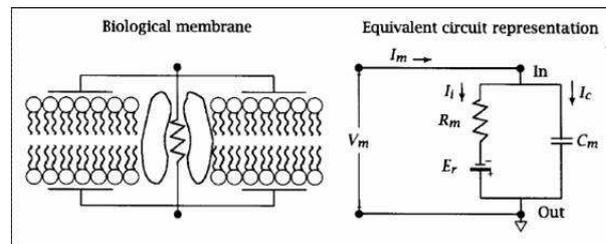
*The strength of general anesthetics is proportional to their solubility in lipids.*

Likewise, all substances dissolvable in lipids act as anesthetics proportional to their solubility. One would assume that this old finding is easily incorporated in the modern understanding of how nerves work, since nerves are where anesthetics function. However, this is not the case! So let us examine why that is, by taking a look on nerve models.

Nerve models are models that explain how nerves transmit a signal from one end to the other. Nerves are cells and as such share characteristics with other cells. Nerves have an outer membrane that confines the cell, inside are DNA, mitochondria, and other organelles.<sup>1</sup> For nerve models, the most important feature is the cell membrane which is made of a lipid bilayer with embedded and adhered proteins. The important question is, whether it is the membrane, or the proteins embedded in the membrane, that are responsible for the function of nerves - the nerve signal.



<sup>1</sup>Picture of nerve from: Nerve Cells and Neurotransmission. Retrieved 12. October, 2006, from [http://teens.drugabuse.gov/mom/tg\\_nerves.asp](http://teens.drugabuse.gov/mom/tg_nerves.asp). Picture of membrane by T. Heimburg made with POV-Ray.



The field of nerves is dominated by one model: The Hodgkin-Huxley Model (HHM). The HHM was developed in the early 1950s, and has since the '60s been regarded as *the* model by a majority of the scientific community.<sup>2</sup> The basic concept of the HHM is that proteins embedded in the nerve membrane act as channels, which transport ions from one side of the membrane to the other. There are specific channels for specific ions, and it is the ion concentration on each side of the membrane that determines, if the transport flows from inside the cell to outside or vice versa. The flow of ions, which have electrical charges, is formalized by an electric circuit. The electric pathways across the membrane are the potassium channel, the sodium channel, and a leak current, which is ions diffusing through the membrane or other ion channels. The membrane is regarded electrically impenetrable and as such functions as a capacitor. To generate the electric nerve signal, the ion channels open and close according to ion concentrations in- and outside of the membrane and with respect to time.

The electric circuit (Fig. 1) is described by ten coupled differential equations, the primary one describing the circuit:

$$I = C_M \frac{dV}{dt} + g_K(t, V_K) (V - E_K) + g_{Na}(t, V_{Na}) (V - E_{Na}) + g_L (V - E_L) \quad (1)$$

$I$  being the current through and  $C_M$  the capacitance of the membrane,  $V$  the total voltage across the membrane with  $E_X$  representing the potential generated by the difference in  $X$ -ion<sup>3</sup> concentration from the inner to the outer of the membrane, and  $g_X$  the conductance of the associated ion

<sup>2</sup>All pictures of HHM adapted from original article, see Literature list.

<sup>3</sup>L being not an ion but the leak current.

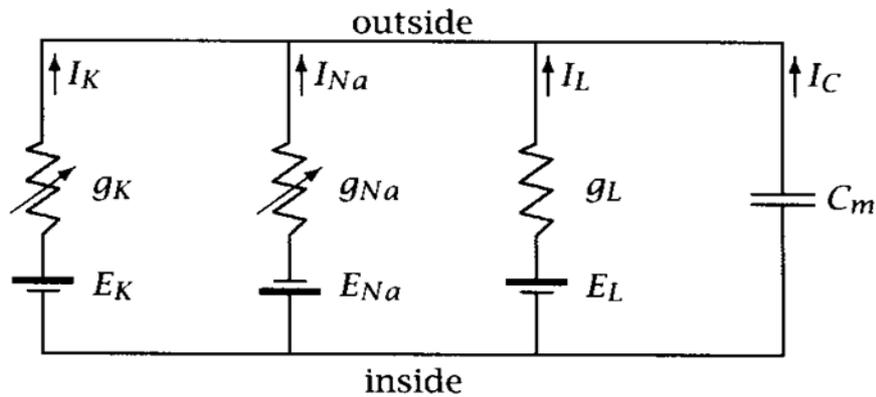


Figure 1: HHM electric circuit.

channel. The  $g_K$  and  $g_{Na}$  are functions of time and voltage in order to make eq. (1) match a measured nerve pulse. In order to replicate a nerve pulse,  $g_K$  and  $g_{Na}$  are governed by a total of nine differential equations that represent 'gating particles' in the membrane.

Evidently, for the nerve signal to be sent, millions of sodium and potassium channels along the nerve will have to open and close in a correlated fashion. And then afterwards, millions of ion pumps will have to work to restore the ion concentrations on each side of the membrane in order for the nerve to be ready for the next signal. This entire process must happen at tremendous speed, since nerves can send signals up to around 120 m/s and up to hundreds of times per second.

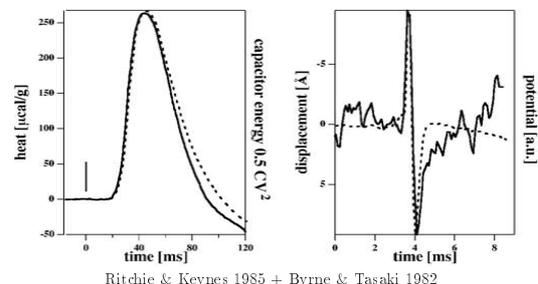
*In the HHM anesthetics act by binding to ion channels, thereby blocking them and the nerve pulse.*

Binding to the channels is suggested to be aided by proteins surrounding the channels, but nevertheless binding sites for *all* anesthetics are required to explain the sedative action! Even if binding sites existed for all anesthetics, it would still be necessary to explain how these can have binding affinities that are proportional to the solubility in oil as the Overton data implies.

The difficulties in explaining the action of anesthetics in the HHM is

not even the most serious problem for the model. In the '80s experiments suggested that many assumptions, on which the model relied, could not be true. For one, Burne & Tasaki measured that when a nerve signal passed a measuring point it was not only electrical but also accompanied by a thickening of the membrane, this contradicts the assumption in the HHM that the membrane has a constant capacitance, since capacitance is reversely proportional to distance of capacitor plates – the membrane boundaries. But worse, Ritchie & Keynes also measured that the nerve signal was accompanied by a heat release shortly followed by a heat uptake of roughly the same size.

The rapid energy release and uptake is a clear indication that the nerve signal is a reversible process and therefore consumes (ideally) no energy. This is in clear violation with HHM, which uses energy to both make the electric pulse and to 'reset' the system by maintaining the ion concentrations.



Ritchie & Keynes 1985 + Byrne & Tasaki 1982

It is ironic that Hodgkin and Huxley actually were the first to publish an article (1945), which, rather than channel based action, suggested that the key property of the nerve signal might be a density pulse in the membrane. This even before their popular HHM. That basic idea was revisited and formalized in 2005 by T. Heimburg, leader of MBG, and A.D. Jackson, from NBI, into a ready model: The Soliton Model (SM).

The SM proposes the nerve signal to be a soliton density wave in the nerve membrane.

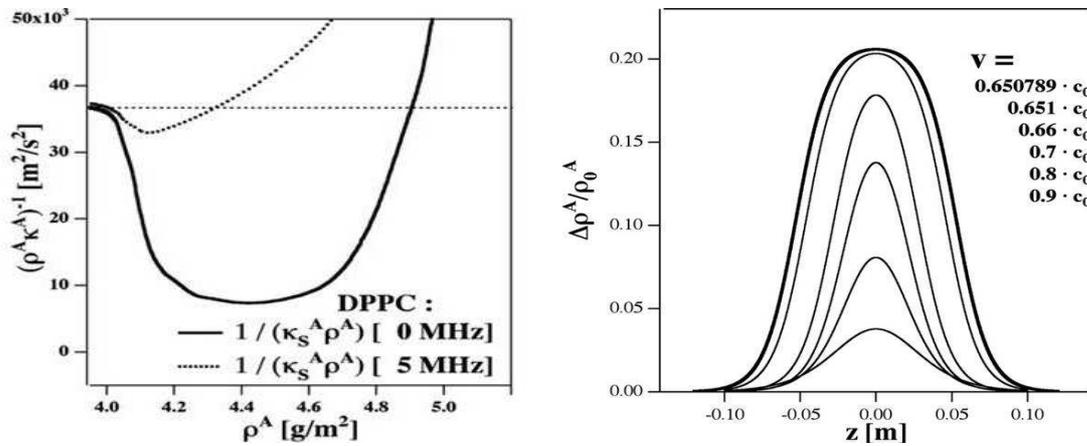
*A soliton is a density wave that does not change shape or velocity as it travels, and therefore loses no energy.*

Already here the SM matches the previously mentioned experiments. The electric signal measured with the nerve signal originates from charged lipids in the membrane, when the density wave passes an area of the nerve membrane, the density of charged lipids increases and changes the electric field, thereby generating an electric signal.

The soliton equation is a wave equation with a dispersion term:

$$\frac{\partial^2}{\partial t^2} \Delta\rho = \frac{\partial \Delta\rho}{\partial x} \left( \frac{1}{\kappa_S^A \rho} \frac{\partial \Delta\rho}{\partial x} \right) - h \frac{\partial^4}{\partial x^4} \Delta\rho \quad (2)$$

where  $\rho$  is the membrane density,  $\kappa_S^A$  is the isentropic area compressibility<sup>4</sup> of the membrane, and  $h$  a dispersion constant. The *key feature* is that  $\kappa_S^A$  displays nonlinear properties around the *melting point* of a bio-membrane, this enable solitons to propagate. (Figure 2)



**Figure 2:** Non-linear properties of the nerve membrane in phase transition regime (picture left) sustain stable density waves: Solitons. (picture right) Taken from Heimburg and Jackson 2005

The link between the Overton findings (which states that the solubility of a drug in lipids - the membrane - decides the anesthetic strength) and the Soliton Model (which key feature is non-linear properties of the compressibility in melting regime) is: Melting point depression.

$$\Delta T = T_m - T = -\frac{RT^2}{\Delta H_M} x_A \quad (3)$$

This simple relation states that the depression of the melting point of a given substance,  $M$ , is directly proportional to the concentration,  $x$ , of a substance,  $A$ , which is solely dissolved in one phase of  $M$ .<sup>5</sup>  $R$  is

<sup>4</sup>A measure of elasticity.

<sup>5</sup>Regarding our experiments,  $A$  is the anesthetic that dissolves only in the liquid phase of the membrane  $M$ , thereby lowering the melting temperature.

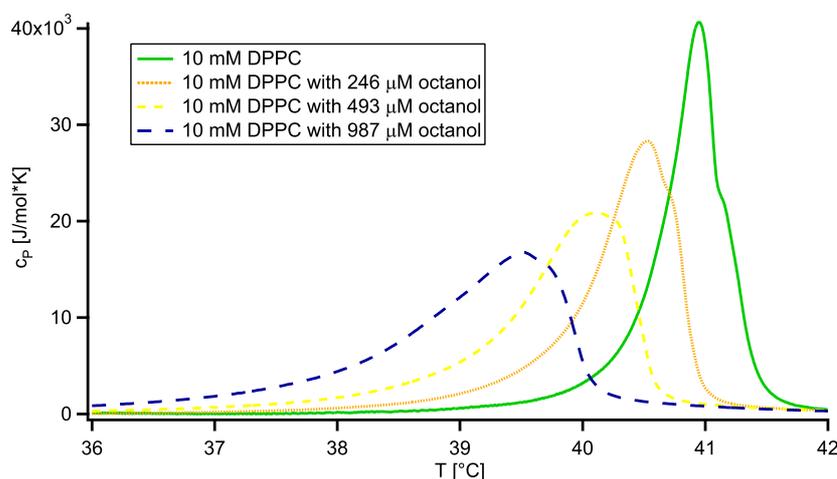
the gas constant, and  $\Delta H_M$  is the melting enthalpy of  $M$ . This physical mechanism is for example responsible for melting ice when salt is added; in other words - since sodium and chloride ions are only dissolvable in the liquid phase of water, ice will melt at a lower temperature to dissolve the ions. Likewise, *every* anesthetic changes the melting point of nerve membranes, thereby changing the properties of the compressibility, and so affecting the solitons which carry the nerve signal!

In the MBG we investigate whether the assumption of melting point depression is viable in a bio-membrane, and how it affects the compressibility. These experiments should firmly establish that the SM fit the findings of Overton, and thereby incorporates anesthetic action better and more simple than any other nerve model.

Our experiments are conducted on a MicroCal Differential Scanning Calorimeter using pure DPPC-lipid membranes<sup>6</sup> and 1-octanol as anesthetic. The goal is to obtain heat capacity profiles of DPPC with various amounts of anesthetic. Our preliminary data shows how anesthetics perform melting point depression on a lipid membrane (Figure 3). When fitting to the theory of melting point depression, the correlation coefficient becomes  $r^2 = 0.998$ .



By author and POV-Ray.



**Figure 3:** Heat capacity of DPPC with varying anesthetic concentration.

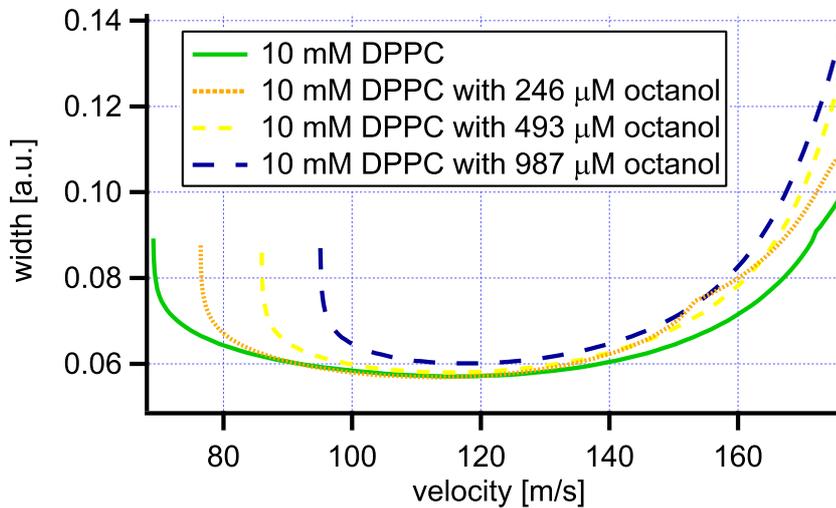
To obtain solitons from data, the isentropic area compressibility,  $\kappa_S^A$ ,

<sup>6</sup>As much as 70% of biological membranes are made of DPPC-lipids.

of the system is calculated from heat capacity via:

$$\kappa_S^A = \kappa_{T,0}^A + \frac{\gamma_A^2 T}{\langle A \rangle} \Delta c_P - \frac{T}{\langle A \rangle c_P} \left( \frac{dA}{dT} \right)_P^2 \quad (4)$$

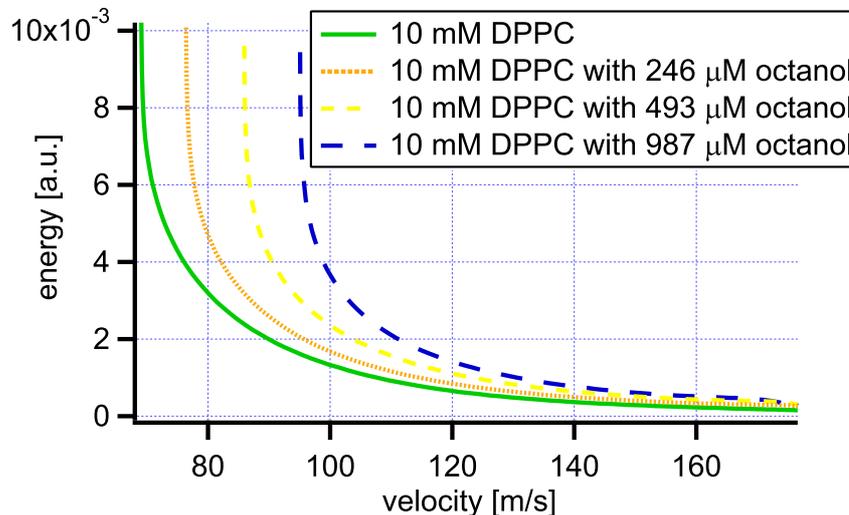
Where on the right side of the equation everything but temperature,  $T$ , and heat capacity,  $c_P$ , are membrane constants. From the derived compressibility, solitons are calculated using the soliton equation. As apparent from Figure 2 on solitons, soliton shape is dependent on the velocity of the soliton. Using our data, half width and energy profiles as function of velocity has been calculated for the different anesthetic concentrations.



**Figure 4:** The half width of solitons as a function of soliton velocity.

What most organisms expect from their nerves, is for them to quickly send the most information possible from one end to the other. To do this requires an optimization of three factors. Firstly, the nerve signal must travel fast. Secondly, the nerve signal must be short, so that many signals may be sent within short time. Thirdly, the signal must be distinct in order to avoid confusion about, whether a signal has occurred or not. A distinct signal is equivalent to high energy of the soliton, but a higher energy of a soliton results in a lower velocity (as apparent from Figure 5). To optimize the nerve signal, a compromise between velocity and energy is required. The boundaries of this compromise is given by Figure

4, which indicates that the soliton width is minimum (i.e. the nerve signal is shortest) between 100-140  $m/s$ . If solitons travel within 100-140



**Figure 5:** Energy of solitons as a function of soliton velocity.

$m/s$ , it can be observed from Figure 5 that the energy costs in the most anesthetized DPPC model membrane are twice that of the model with no anesthetic. So data reveals that it costs more energy to send a soliton in an anesthetized membrane. If that energy is not available, no soliton can be sent!

The beauty of this result is that it is not dependent on which anesthetic being used, but only the change in heat capacity! The change in heat capacity should by the principle of melting point transition be caused by any membrane penetrating agent according to solubility, and so fulfill the 100 year old finding of C.E. Overton!

*To sum up: Problems with the current view on how nerves signal were discussed. A new model on nerve function has been introduced, and it was shown how this new model easily incorporates the action of anesthetics.*

We are currently doing experiments with many types of anesthetics, to firmly establish the results presented in this article. In addition, we are calculating how much energy is available in membranes in order to predict

when anesthesia occurs. Moreover, we conduct Monte-Carlo simulations of lipid membranes adding anesthetics. The results are planned to be presented in a master thesis by the spring 2007.

*The Membrane Biophysics Group is located at the Niels Bohr Institute at Blegdamsvej; currently there are twelve associated members including bachelor, master, Ph.D, and Post Doc. students. Other research areas than anesthetics and the Soliton model are permeability of membranes and membrane enzymes. In connection to the Soliton model, the MBG is preparing to observe the nerve pulse in artificial and real nervous systems in the near future. For more information contact head of MBG Thomas Heimburg, [theimbu@nbi.dk](mailto:theimbu@nbi.dk), or author of this article Kaare Græsbøll, [grasboll@nbi.dk](mailto:grasboll@nbi.dk).*

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- [6]T. Heimburg and A.D. Jackson, PNAS, vol **102**, no 28, 9790-9795, 2005
- [7] The Persistence of Vision Raytracer, or POV-Ray, is a ray tracing program available for a variety of computer platforms.  
<http://www.povray.org/>