ECRONICON OPEN ACCESS

NEUROLOGY
Research Article

Differential Actions of Volatile Anaesthetics and a Systemic Barbiturate on Strongly Electrically Coupled Neurons

Munir M. Qazzaz[1] and William Winlow[2]*

[1]Department of Nursing, Pharmacy and Health Professions, University of Birzeit, Palestine

[2]Department of Biology, University of Naples Federico II, Naples, Italy and Department of Veterinary Preclinical Sciences, University of Liverpool, UK

***Corresponding Author:** William Winlow, Department of Biology, University of Naples Federico II, Naples, Italy and Department of Veterinary Preclinical Sciences, University of Liverpool, UK.

Received: November 18, 2015; **Published:** November 21, 2015

Abstract

The actions of clinical concentrations of volatile anaesthetic halothane and isoflurane were compared with those of pentobarbitone on identified neurons of the pond snail Lymnaea stagnalis. Application of halothane and isoflurane (1% v/v and 2% v/v), to the CNS of Lymnaea caused the strongly electrically coupled neurons VD1 and RPD2 to become quiescent, with a significant increase in membrane potential and a significant reduction in their coupling coefficient.

The effects of volatile anaesthetics were dose dependent, and the actions of halothane were more rapid than those of isoflurane, reflecting their different anaesthetic potencies. Sodium-pentobarbitone had markedly different effects from the volatile anaesthetics. Clinical concentrations of pentobarbitone (100 ± M) caused VD1 and RPD2 to become quiescent with no significant change in either their resting membrane potential or the coupling coefficient between them. Higher concentrations of pentobarbitone (0.5 and 2.0 mM), caused VD1 and RPD2 to fire in doublets and triplets until their hyper-activity increased leading to paroxysmal depolarising shifts (PDS) (phase I). Following this the cells became quiescent and hyperpolarised (phase II). During phase I, the VD1-RPD2 coupling coefficient increased significantly, but during phase II the coupling coefficient returned to control values. There was no dose dependency in the effects of pentobarbitone at high concentrations.

These results clearly demonstrate that the volatile anaesthetics and barbiturates have different mechanisms of action at electrical synapses.

Keywords: *Volatile anaesthetics; Barbiturates; Identified neurons; Lymnaea; Electrical coupling*

Introduction

The cellular mechanisms of general anaesthesia are still being elucidated because, unlike many other drugs that produce an effect via a single mechanism, anaesthetics have non-specific pharmacological actions, affecting different systems including, ionic currents, receptors and second messengers [1-6]. At present, it is not clear which cellular effects are important for the various anaesthetic responses (unconsciousness, amnesia, analgesia, etc) and which of them contribute only to unwanted side effects (e.g. respiratory depression, central and peripheral depression of the cardiovascular system and malignant hypothermia [7]). However it has become very apparent that one anaesthetic cannot necessarily simply be replaced by another [8]. This is why we compare here the effects of volatile anaesthetics with an intravenous barbiturate, pentobarbitone (pentobarbital).

Using the advantageous mollusc an preparation Lymnaea stagnalis as a model for anaesthetics research, halothane was found to have differential effects on the spontaneous firing of different neurons. Some neurons were found to become quiescent while others exhibited

paroxysmal depolarising shifts (PDS) after exposure to either clinical or high concentrations of halothane [9,10]. The systemic anaesthetic, pentobarbitone, also caused different effects on the spontaneous activity of different neurons [11]. Several studies of anaesthetic actions have also been carried on Lymnaea chemical synapses both on vitro and in culture [12-18], but except for the effects of anaesthetics on weakly electrically coupled neurons in Lymnaea [19] only preliminary studies have been performed on electrical synapses in this preparation [20,21]. However, Terrar and Victory have shown that halothane and isoflurane uncouple isolated pairs of guinea-pig ventricular muscle cells [22] and halothane and ethrane (enflurane) are known to reversibly reduce dye coupling between cultured neonatal rat cardiac myocytes in a dose dependent manner [23]. Furthermore, electrical synapses are believed to be less sensitive to most anaesthetics than are chemical synapses [24-26].

According to Bennett "gap junctions are the morphological substrate of one class of electrical synapse", and it is this class of electrical junctions that is under consideration here [26]. As recently as 2002 gap junctions were supposed to be of little importance in mammals [28]. However, the ubiquity of gap junctions in the mammalian central nervous system has now been established [29] alongside those already known in invertebrates and cold blooded vertebrates.

Many studies on electrical synapses have been carried out on dissociated cells in culture [25,26,30], including dissociated myocardial cells [23] as mentioned above. It is interesting to note that the gap junction protein in heart muscle is indistinguishable from that of astrocytes [31]. However it is difficult to study electrical synapses in situ in the mammalian Brain and Lymnaea stagnalis presents us with a tractable, simpler system to facilitate these studies: the strongly electrically connected, giant neurons VD1 and RPD2 [32].

VD1 and RPD2 fire in almost perfect synchrony, suggesting that they function as one unit. Janse., *et al*. [32].and de Vlieger., *et al*. investigated the origin of the spiking activity in this system by making intracellular recordings before and after culturing these two neurons separately [33, 34]. They showed that VD1 fired spontaneously while RPD2 was almost quiescent, leading to the suggestion that, in the intact CNS, VD1 may act as a driver neuron whilst RPD2 is a follower neuron. In other words VD1 is the pacemaker in this system. These findings were confirmed when they used a hybrid current/voltage clamp technique. Janse., *et al*. [33]. demonstrated that the VD1 /RPD2 system receives excitatory and inhibitory synaptic input and found that the system modulates the animal's cardio-respiratory functions [33,35].

Dye coupling is one of the accepted methods to demonstrate coupling between cells in different tissues. It has been reported that many low molecular weight, high fluorescence efficiency and low toxicity dyes, such as Lucifer Yellow, can pass readily through low resistance junctions between cells in different tissues [36-38]. In spite of these results, Audesirk., *et al*. [39]. demonstrated that Lucifer Yellow failed to pass through the junction between neurons VD1 and RPD2 of Lymnaea even though these neurons are known to be strongly electrically coupled [39]. Soffe and Benjamin suggested that the site of the electrotonic junction between these two neurons is located not far from the cell bodies in the viscero-right parietal commissure between the visceral and the right parietal ganglia, although they recognised that an electron microscopical study was needed for confirmation [40].

An electrophysiological method to measure the strength of the connection between coupled cells was first used by Bennett [41]. In brief he applied sub-threshold current pulses to one neuron and recorded their effects in the second neuron and calculated the ratio of the voltage changes across the membrane of both neurons. Strongly but distantly coupled cells such as the cerebral giant cells (CGC's) of Lymnaea [42], have cell bodies so far apart that the injection of any sub-threshold currents into the cell body of one neuron will cause no change in the soma membrane potential of the other neuron and are thus inappropriate for a detailed study of the effects of general anaesthetics on the coupling coefficient. However, this method is appropriate for calculating the coupling coefficient in cells such as VD1/RPD2 which lie close to each other (Figure 1) although the coupling coefficient will always be something of an underestimate due to the space constant of the two neurons.

Here we compare the effects of the volatile anaesthetics, halothane and isoflurane, (1% and 2%) with those of the laboratory animal anaesthetic pentobarbitone (100 ± M, 0.5 and 2.0 mM) on the neurons VD1 and RPD2 of the pond snail Lymnaea stagnalis. We also demonstrate in detail the time course of the onset and offset of the anaesthetic actions.

Materials and Methods

The methods used are as previously reported [32]. Briefly, simultaneous intracellular recordings were made from the VD1 and RPD2, using bridge balance Neurology NL102 preamplifiers. These recordings were performed when the snail brain was perfused with HEPES buffered saline (HBS), or HBS-containing the anaesthetic agents. Data were captured and transferred to a PC using a C.E.D. 1401 interface and analysed using a spike2 software package. The coupling coefficients of the neurons were determined by the method shown in Figure 1.

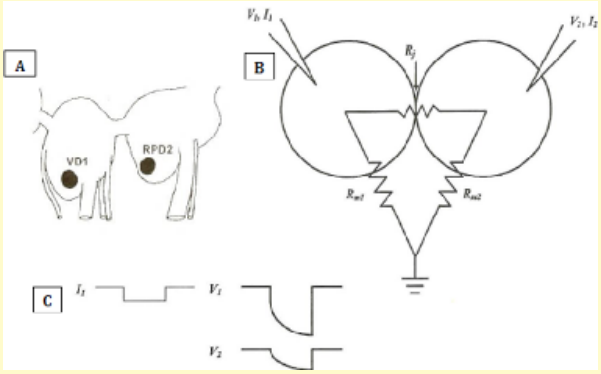

Figure 1: A) The locations of the tightly electrically coupled giant neurons VD1 (visceral dorsal 1 and RPD2 (right parietal dorsal 2), each 100-200 µm in diameter, in the median visceral and right parietal ganglia of Lymnaea B) Electrical equivalent circuit, adapted from [43] representing the two high resistance electrically coupled cells (R_{m1} and R_{m2}) which are electrically coupled via a low resistance junction (Rj). Each cell is impaled using a glass microelectrode filled with 2 mM K_2SO_4 and used C) to inject current (I_1 or I_2) and to simultaneously record membrane voltage (V_1 and V_2) from which the coupling coefficient is calculated.

4-mM pentobarbitone (Sigma) was prepared in HBS, as a stock solution which was kept in a refrigerator and renewed after a maximum period of one week. When required the stock solution was diluted with HBS to give three working solutions with concentrations of 100 ± M 0.5 mM and 2 mM. Two different concentrations of each of the volatile anaesthetics, halothane and isoflurane were used. They were prepared with the aid of vaporisers (Fluotec 3 for halothane and Isotec 3 for isoflurane, Ohmeda). The length of the plastic tubing between the vaporisers and the glass bottles was minimised to reduce any possible absorption of the anaesthetics. The flow rate of air was adjusted to 2.5 litres per minute and the concentration of the anaesthetic was adjusted to the required concentration. The vaporised anaesthetic was bubbled into the HBS through a filter stone and the gas was allowed to equilibrate for 20 minutes at ambient temperature. The excess of the anaesthetic was drawn off into a fume cupboard [38, 44]. The pH of the HBS -was kept constant at 7.9. The effect of different anaesthetic agents on the pH was investigated. Neither volatile nor barbiturate anaesthetics, at any of the concentrations used, had any effect on the bath pH (n = 3 for each anaesthetic agent at each concentration).

All anaesthetic solutions were prepared immediately prior to the experiment. Different flasks, tubing and filters were used for different anaesthetic to minimise the risk of contamination. The concentrations of the anaesthetics quoted in the text are the initial reservoir concentrations. However, various concentrations (in mM) of the two volatile anaesthetics present in the recording dish were determined by extracting the anaesthetic into carbon tetrachloride and analysed using gas liquid chromatography (GLC). The final measurement concentrations were as in table 1 and this compares well with previously recorded values in this laboratory [45-47] and are well within the clinical range (0.20-0.78 mM) [48] for halothane.

	Concentrations in flasks		Concentration in recording dish
Anaesthetic	%	mM (m ± sd)	mM (m ± sd)
Halothane	1	0.71 ± 0.05	0.43 ± 0.07
(n = 4)	2	1.14 ± 0.08	0.77 ± 0.10
Isoflurane	1	0.65 ± 0.09	0.37 ± 0.05
(n = 4)	2	1.2 ± 0.12	0.78 ± 0.09

Table 1: *Comparison between anaesthetic concentrations in the flasks and the recording dish using gas liquid chromatography.*

To investigate the effect of general anaesthetics on the coupling coefficient between VD1 and RPD2 neurons, the following procedure was used: Preparations were kept perfused with HBS and were left to stabilise for at least 30 min. Simultaneous intracellular recordings from VD1 and RPD2 were then made. Hyperpolarising current pulses (0.1 nA) were injected into VD1 for 1-2 seconds every 5-10 seconds and were kept constant throughout the experiments. The coupling coefficient between VD1 and RPD2 was calculated according to the method of Bennett [41]. However, in some experiments the hyperpolarising current pulses were injected into RPD2 rather than VD1. The preparation was then perfused with HBS-containing 1% halothane where its effect on the coupling coefficient was recorded. The preparation was washed until it recovered from the effect of halothane and returned to its normal activity and again the coupling coefficient was calculated. The whole procedure was then repeated with 2% halothane, 1% and 2% isoflurane and in some experiments where possible it was repeated with 100 ± M, 0.5 and 2.0 mM pentobarbitone. In some experiments the application of anaesthetic HBS were carried out in a different sequence again with washes in between. The results were not significantly different, regardless of the sequence used.

Results

In isolated whole brain preparations, the neurons VD1 and RPD2 were usually (11/12) found to fire spontaneously and generated type 2 action potentials with a distinct calcium plateau (Figure 2), according to nomenclature of Benjamin and Winlow [31]. Hyperpolarising current pulses (0.1 nA) were injected into the VD1 neuron to calculate the coupling coefficient between VD1 and RPD2. However, in a few experiments (n = 3), pulses were injected into RPD2 neuron to check if there was any rectification at the electrical synapse between these neurons. Regardless of which cell was injected with current, there was no difference in the coupling coefficient between VD1 and RPD2 (Figure 3). In most experiments, the measurement of the coupling coefficient between these two neurons was calculated by applying the Hyperpolarising current pulse to VD1 neuron since we know that it is the pacemaker neuron in this system [33].

Effects of general anaesthetics on spontaneous electrical activity and coupling coefficient between Lymnaea neurons

a) Halothane

The effects of halothane on VD1/RPD2 were examined in 12 different preparations. Different concentrations of halothane (1% and 2%) were applied to the bath solution as previously described. With 1% halothane the neurons VD1 and RPD2 became quiescent after 6.9 ± 0.8 minutes. Their resting membrane potential, originally was -54 ± 5.7 mV, and after the anaesthetic became hyperpolarised to -87 ± 4.4 mV. When 2% halothane was applied, it affected these two neurons more rapidly than 1% halothane (Table 2). The neurons became quiescent after 3.4 ± 0.6 minutes, and were hyperpolarised to the same membrane potential at both concentrations (Figure 4).

The time to maximum effect (Tm) is the time taken by the anaesthetic to have its maximal effect on VD1 and RPD2 neurons (Figure 4). The recovery time (Tr) is the time it takes for the preparation to resume its normal electrical activity. Although Tm of both 2% halothane and 2% isoflurane was approximately 50% shorter than 1% the Tr for 2% halothane and isoflurane was approximately 30% longer than 1%. Furthermore, the higher the pentobarbitone concentration the shorter the Tm and the longer the recovery time.

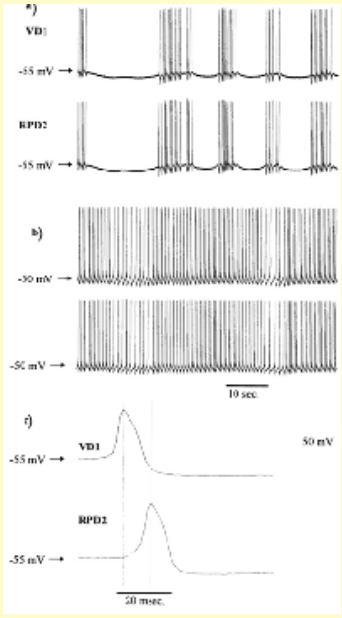

Figure 2: *Spontaneous firing of the neurons VD1 and RPD2. The spontaneous firing of the peptidergic neurons VD1 and RPD2 of Lymnaea was either (a) patterned with quiescent periods between them or (b) regular firing with a frequency 0.2-0.6 Hz. (c) shows that VD1 is the pacemaker for these two cells and that RPD2 fires after a delay of 8.3 ± 2.5 msec. (n = 6), confirming the data of Janse., et al. and de Vlieger., et al. [33, 34].*

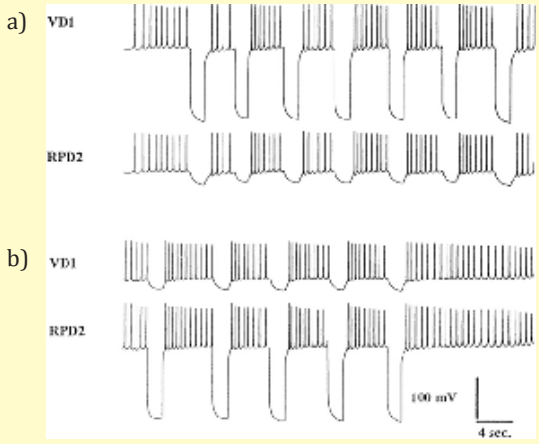

Figure 3: *Calculating the coupling coefficient between VD1 and RPD2 by applying the hyperpolarising current to either VD1 or RPD2. This figure shows that the coupling coefficient is not affected by the application of hyperpolarising current pulses to either VD1 or RPD2. This means that there is no rectification in the electrical synapse between these two neurons. a) When the hyperpolarising current was applied to VD1, the coupling coefficient was found to be 26.5%. b) Similar results were gained when the same hyperpolarising current was applied to RPD2 (26.8%). This experiment was repeated 3 times, on three separate preparations with similar results.*

Anaesthetic	Tm (min) (mean ± S.D.)	Tr (min.) (mean ± S.D.)
1% halothane	6.9 ± 0.8 (5)	8.3 ± 1.5
2% halothane	3.4 ± 0.6 (5)	11.1 ± 1.3
1% isoflurane	10.3 ± 0.5 (5)	5.9 ± 1.1
2% isoflurane	5.4 ± 1.0 (5)	8.8 ± 1.4
0.1 mM pentobarbitone	5.8 ± 0.9 (6)	10.7 ± 0.9
0.5 mM pentobarbitone	2.6 ± 1.2 (6)	23.1 ± 2.8
2.0 mM pentobarbitone	1.6 ± 0.6 (6)	26.6 ± 2.7

Table 2: *The times to maximum effect (Tm) and recovery times (Tr) of general anaesthetics on VD1 and RPD2 neurons (n = 12).*

During the controls and washes the coupling coefficient was equal to 26 ± 0.91% (n = 8). The application of HBS-containing 1% halothane showed a significant reduction in the coupling coefficient 6.81 ± 1.24%. 2% halothane decreased the coupling coefficient even more (3.88 ± 0.93%) (Figures 5,6). However, this effect was completely reversible and the original coupling coefficient was regained after washing with HBS.

b) Isoflurane

The effect of the volatile anaesthetic isoflurane on the spontaneous electrical activity of different Lymnaea neurons has been examined on 12 different preparations. On perfusion of the preparations with 1% isoflurane the spontaneous firing of VD1/RPD2 declined and eventually ceased after 10.3 ± 0.5 minutes. With 2% isoflurane similar results were seen but the effect was faster (table 2). Both concentrations of isoflurane were found to affect the resting membrane potential. Neurons were hyperpolarised from a resting membrane potential of -52 ± 7.6 mV to -84 ± 5.1 mV.

The value of the coupling coefficient between VD1 and RPD2 neurons was 26.3 ± 1.12% (n = 8) during controls and Isoflurane decreased the coupling coefficient significantly in a dose dependent manner. 1% reduced it to 15.98 ± 1.82% and 2% reduced it to 9.43 ± 0.79%. In both concentrations the effect was completely reversible (Figure 6).

c) Na-pentobarbitone

Na-pentobarbitone is an intravenous (iv) general anaesthetic from the barbiturate family. The differential effects of pentobarbitone have been studied on different ionic channels and the spontaneous electrical activity of many molluscan neurons [11]. Three different concentrations of pentobarbitone were used to investigate its effect on the coupling coefficient between neurons (n = 10). One concentration was within the clinical range (100 ± M) to demonstrate the effect of clinical doses of pentobarbitone (Figure 7). However, the other two concentrations were higher than the usual clinical range (0.5 mM and 2.0 mM) although these concentrations have been used clinically for neuro-protective purposes.

Fresh preparations of Lymnaea CNS (n = 14) were perfused with HBS for controls and with HBS-containing pentobarbitone at different concentrations to investigate its effects on the spontaneous electrical activity of VD1 and RPD2 neurons. Using 100 ± M pentobarbitone, some neurons become quiescent. However, higher concentrations of barbiturates elicited different effects on the same neuron. When 100 ± M pentobarbitone was applied (n = 6) VD1 and RPD2 neurons became quiescent after 5.8 ± 0.9 min. with a samall average increase in resting membrane potential from -57 ± 2.1 mV to –61 ± 0.8 mV (Figure 7). This effect was reversible, and they resumed their normal electrical activity after 10.7 ± 0.9 min. from starting perfusion of the preparations with HBS (Table 2).

After perfusing the CNS with HBS-containing 100 ± M pentobarbitone the cells showed no significant change in coupling coefficient, which was 26.97 ± 3.07% during controls and 26.49 ± 2.41% during the test period (Figure 8 and 11).

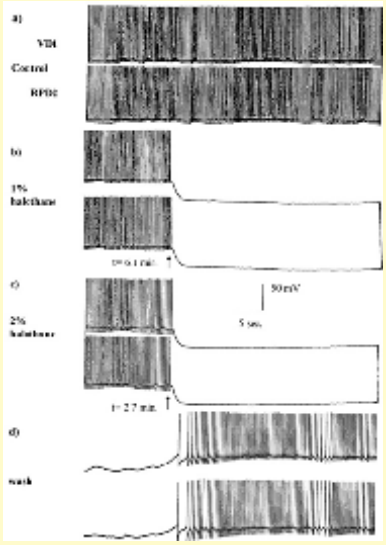

Figure 4: *Effect of halothane on the spontaneous electrical activity of VD1 and RPD2 neurons. a) When the brain was perfused with HBS normal electrical activity of VD1 and RPD2 was maintained. b) Perfusing the CNS with 1% halothane caused the cells to become quiescent. c) The onset of quiescence was more rapid in 2% halothane (2.7 min) than in 1% halothane (6.1 min) in this preparation (t = time after application). However, in both cases the resting membrane potential was reduced from -55mV to -90 mV. d) The effect of halothane at both concentrations was completely reversible.*

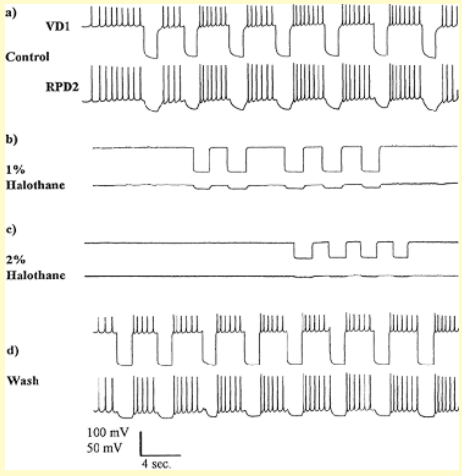

Figure 5: *Effect of halothane on the coupling coefficient between VD1 and RPD2 neurons. During controls where the CNS was perfused with HBS, the coupling coefficient was 26.0 ± 0.91% (n = 8). b) When the CNS was perfused with HBS-containing 1% halothane, the coupling coefficient was significantly reduced to 6.81 ± 1.24%. c) Coupling was further reduced (3.88 ± 0.93%) when HBS-containing 2% halothane was applied. d) The effect of halothane was reversed when the CNS was washed with HBS for 15 min.*

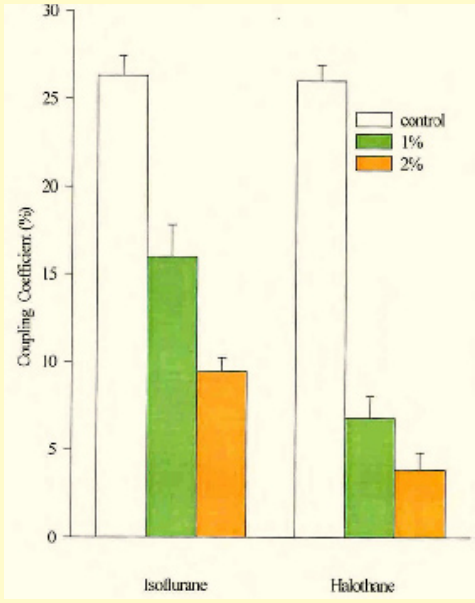

Figure 6: *Summary of the Effect of volatile anaesthetics on the coupling coefficient between VD1and RPD2. The coupling coefficient was significantly decreased by 1% and 2% isoflurane (n = 8) in a dose dependent manner. 1% and 2% halothane induced an even greater reduction in the coupling coefficient in comparison with isoflurane. Bars present mean ± SE.*

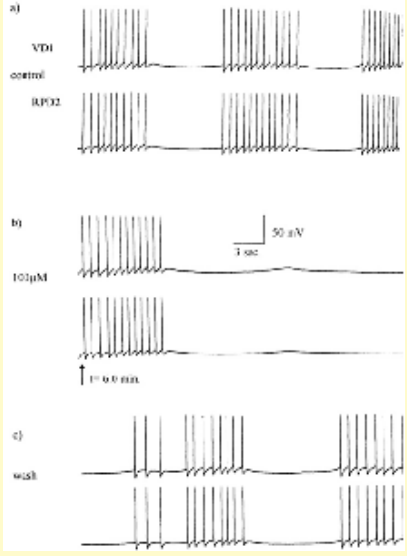

Figure 7: *Effect of a clinical concentration of pentobarbitone (100 ± M) on the electrical activity of VD1 and RPD2 neurons. a) Spontaneous synchronous firing of the neurons VD1 and RPD2. The resting membrane potential is -58 mV in both neurons. b) After perfusing the brain with 100 ± M pentobarbitone, the cells became quiescent with an increase in the membrane potential (-67 mV). c) The effect of the clinical concentration of pentobarbitone (100 ± M) was completely reversible and the cells returned to their normal firing pattern.*

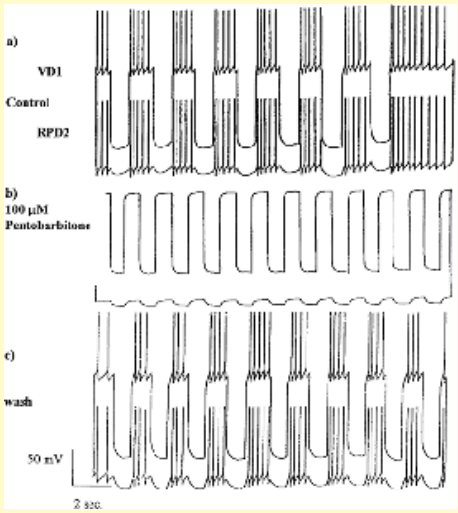

Figure 8: *Effect of a clinical concentration of pentobarbitone (100 ± M) on the coupling coefficient between VD1 and RPD2 neurons. The application of a clinical concentration of pentobarbitone (100 ± M) to the CNS demonstrated no significant change in the coupling coefficient between the neurons VD1 and RPD2. During controls and washes coupling was 26.49 ± 2.41% whereas when the CNS was perfused with HBS-containing pentobarbitone (100 ± M) the coupling coefficient was 26.97 ± 3.07%.*

In contrast, the application of 0.5 mM pentobarbitone (n = 8) caused these same neurons to go through two distinct phases. In phase I there was an overall increased spike frequency, which rapidly changed to doublet and triplet firing, and by the end of the phase changed to paroxysmal depolarising shifts (PDS). In phase II the resting membrane potential of these neurons was increased from -50 ± 3.5 mV to -85 ± 4.3 mV, leading to quiescence.

The coupling coefficient between the neurons VD1 and RPD2 increased significantly in phase I when 0.5mM pentobarbitone (n = 10) was applied (56.72 ± 5.43%). However, during phase II the cells went quiescent with no significant change in their coupling coefficient as compared to normal. Control values of 26.97 ± 3.07% changed to 27.28 ± 3.66% during phase II (Figure 11).

The same results were seen when the CNS was perfused with HBS-containing 2.0 mM pentobarbitone (n = 8) (Figures 9and 10). The effects of pentobarbitone, at these two concentrations on the spontaneous electrical activity of the neurons VD1 and RPD2 were fully reversible (Table 2). During different experiments, the CNS was perfused with these different concentrations in different sequences and similar data were always obtained.

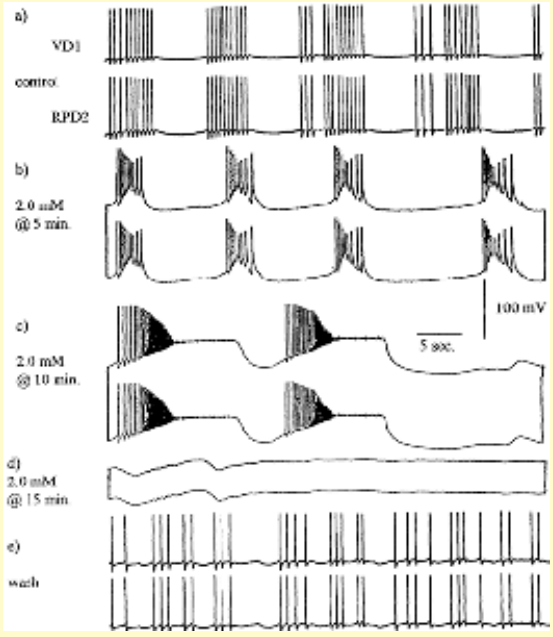

Figure 9: *Effect of 2.0mM Pentobarbitone on the spontaneous electrical activity of the neurons VD1 and RPD2. a.) Normal electrical activity of the electrically coupled neurons VD1 and RPD2. b) After 5 minutes perfusion the brain with HBS-containing 2.0 mM pentobarbitone, VD1 and RPD2 neurons started to fire in bursts. c) Their hyper-activity continued to develop to PDS after 15 minutes. d) After 20 minutes, the neurons became quiescent. e) The effect of 2.0 mM pentobarbitone was completely reversible and the neurons partially returned to their normal activity. The normal resting membrane potential of these neurons was -55 mV which increased to -82 mV with 2.0 mM pentobarbitone.*

There was a clear and distinctive change in the coupling coefficient during the two phases. In phase I, from control values of 24.47 ± 3.54% the coupling coefficient was significantly increased (54.59 ± 3.66%). However, there was no significant difference in the coupling coefficient, as compared to control, during phase II (26.76 ± 3.61%) (Figures 10,11). With these two concentrations (0.5 and 2.0 mM) the effect of pentobarbitone on coupling coefficient showed no dose dependency and its actions were completely reversible (Figure 9).

In two further experiments, the CNS was perfused with HBS containing either 1 or 2% halothane, or 1 or 2% isoflurane, 100 ± M, 0.5 mM or 2.0 mM pentobarbitone in different sequences. The brain was washed between applications of each concentration of each anaesthetic agent with HBS to allow the neurons to resume their normal activity before the application of the next anaesthetic agent and to check if there was any interaction between them. These experiments demonstrated that the effect of the anaesthetics were the same as if they were applied separately and that pre-treatment with another anaesthetic had no effect on its action on the neuronal activity of VD1 and RPD2.

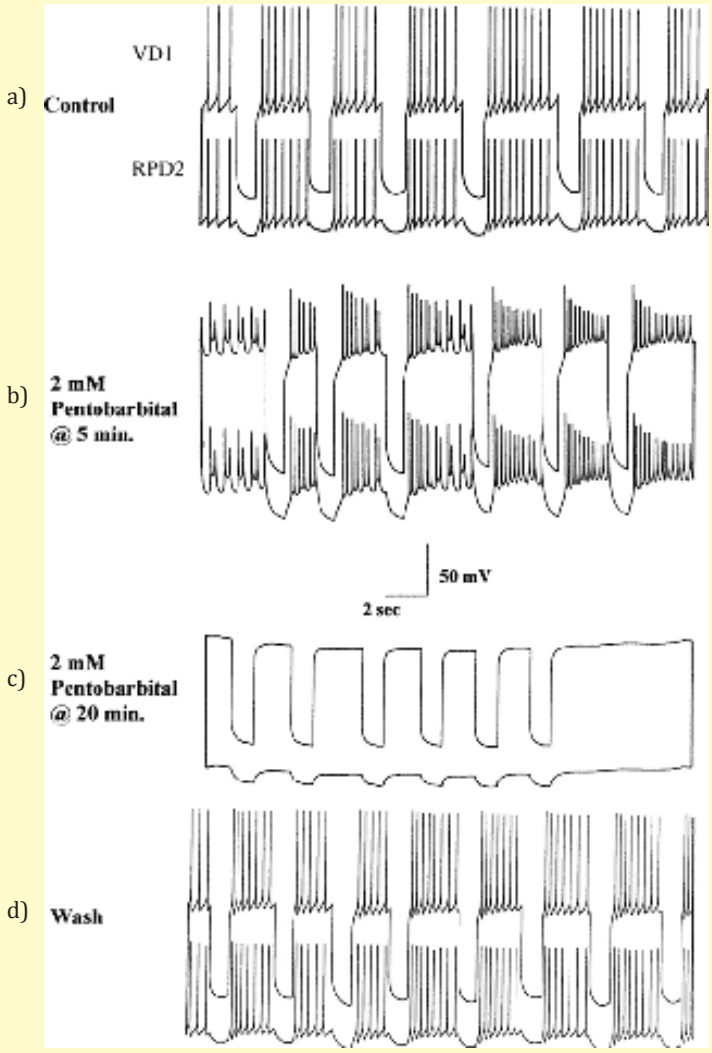

Figure 10: *Effect of 2.0 mM Pentobarbitone on the coupling coefficient between VD1 and RPD2. a) When the brain was perfused with HBS, the coupling coefficient between VD1 and RPD2 and was 26.97 ± 3.07%. b) After the application of HBS-containing 0.5 pentobarbitone and during phase I, their coupling coefficient increased significantly to 56.97 ± 5.443%. c) Interestingly, there was no significant difference, from, control, in the coupling coefficient during phase II (28.28 ± 3.66%). d) after washing the CNS with HBS these neurons returned to their normal activity and to their control coupling coefficient.*

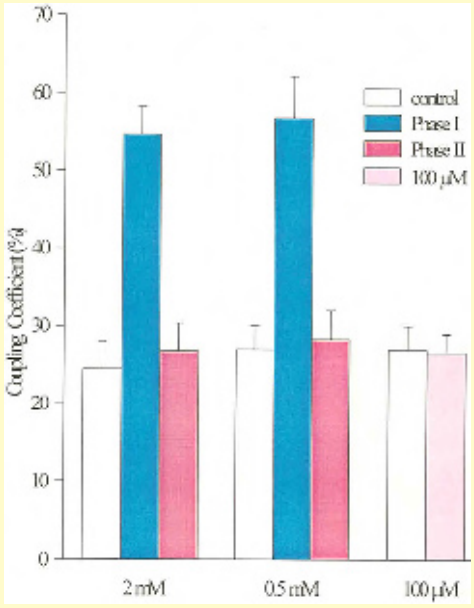

Figure 11: *Effect of barbiturates on the coupling coefficient between VD1 and RPD2. The coupling coefficient between VD1 and RPD2 was significantly increased during the PDS phase induced by both 0.5 and 2.0 mM pentobarbitone (n = 10) but there was no significant change in the second phase when the cells become quiescent, as with 100 µm pentobarbitone. Bars represent mean ± SE.*

Discussion

In this paper we have demonstrated that volatile anaesthetics and barbiturates have clearly different effects on the same neurons. Their actions on the coupling coefficient of the electrically coupled neurons VD1 and RPD2 are such that volatile anaesthetics decrease it in a dose dependent manner, whilst barbiturates either have no effect (at low concentrations) or increase it markedly. Reports elsewhere indicate that a reduction in the coupling coefficient is more likely due to a decline in input resistance and vice versa [4, 49]. Allied to these changes there are clear modifications of membrane time constant and capacitance [4, 49]. Thus it follows that anaesthetics not only modify membrane receptor proteins [50-52] but also have major effects on membrane lipids. The sites of action of general anaesthetics have been a matter of conjecture for many years, but the work presented here makes it clear that there are likely to be direct effects on the membrane lipids. "Whether these actions are produced only at the lipid-protein interface or are generated throughout the membrane lipids remains to be seen" [4].

The actions of general anaesthetics on excitatory and inhibitory chemical synapses are well documented [2,3,8, 53-55]. Almost all authors have agreed that general anaesthetics depress excitatory synapses and enhance inhibitory synapses in different species [3,54-56]. This latter response is supported by the work with GABA [56]. For instance, general anaesthetics depressed excitatory synapses between VD4 and its follower Pedal A (PeA) group neurons of Lymnaea [54,55] and in the guinea pig neocortex [57] but depressed the NMDA, AMPA and Kainate-induced currents in Xenopus laevis oocytes expressing mouse and human brain mRNA [58]. In contrast, they were found to enhance neuronal inhibition (3,54,55); for example they enhanced the actions of inhibitory synapses between Lymnaea neurons [55] and prolonged the inhibitory postsynaptic GABA currents in rat hippocampus CA1 pyramidal cells [59].

However, general anaesthetics affect not only chemical synapses but also electrical synapses. Though, electrical synapses play a vital role in cell-to-cell communication [37,60,61], only recently has interest increased in the actions of general anaesthetics on the gap junctions between cardiac muscle [62-64]; embryonic cells and gametes [38,65,66] and neuronal tissue [20,21,60,61,66-68].

Volatile anaesthetics showed a significant reduction in the coupling coefficient between VD1 and RPD2 neurons and between PeA neurons [20,49,69]. As previously reported, the intracellular calcium concentration [Ca^{2+}] i plays a major role on junctional conductance between cells in different tissues [37,61,70]. Daniell., et al. found that anaesthetics, such as halothane raise [Ca^{2+}] i and they suggested that it was due to its release from intracellular sites [71]. Ahmed and Winlow demonstrated that halothane raises [Ca^{2+}] i in the presence and the absence of extracellular calcium in a dose dependent manner [72]. It was also found that increasing [Ca^{2+}] i causes electrical and dye decoupling between cardiac cells [37,70,73]. Other different factors can cause decoupling as well; lowering [pH]i , neurotransmitter or second messengers or drugs that modulate any of these components. Since general anaesthetics were found to increase the [Ca^{2+}] i this may be the mechanism by which volatile anaesthetics cause decoupling. Since the neuronal decoupling was produced together with a reduction in the input resistance of these neurons [49], this may suggest another pathway through which volatile anaesthetics caused the decoupling. The decrease in input resistance may be explained not only by activation of specific ionic channels but by the disruption of the lipid membrane allowing current to leak out through non-specific channel pores. Therefore, injected subthreshold currents escape to the extracellular space rather than pass through the gap junctions between the two neurons.

The effects of pentobarbitone on the electrical coupling between VD1 and RPD2 cannot be explained using the same hypothesis because it produced different actions. Pentobarbitone, within the clinical concentration demonstrated no change in the coupling coefficient though the action potential was abolished and the cells became quiescent. In contrast, 0.5 and 2.0 mM increased the coupling coefficient during phase I and it returned to control values during phase II. It is most probable that pentobarbitone produces its effect on the coupling coefficient through a different mechanism from volatile anaesthetics. Further studies are in progress to monitor the passive membrane properties of VD1 and RPD2, including input resistance, time constant and membrane capacitance. However, to elucidate these mechanisms, [Ca^{2+}] i needs to be monitored during application of each class of anaesthetic. What is more it is now appreciated that electrical synapses are highly plastic modifiable by a variety of agents, including neurotransmitters [27,29], and subject to block by a wide variety of agents, many of which are non-specific [74]. Thus the effects of anaesthetics on the junctional resistance and the junctional proteins need to be understood.

Conclusions

1. The effects of volatile general anaesthetics and barbiturates on the strong electrical coupling between the Lymnaea giant neurons VD1 and RPD2 are quite different.
2. The volatile anaesthetics halothane and isoflurane reduce the coupling coefficient in a dose-dependent manner at clinical concentrations.
3. Clinical concentrations of sodium pentobarbitone (100 mM) caused VD1 and RPD2 to become quiescent with no significant change in either their resting membrane potential or the coupling coefficient between them.
4. Higher concentrations of pentobarbitone (0.5 and 2.0 mM), caused VD1 and RPD2 to fire in doublets and triplets until their hyper-activity increased leading to paroxysmal depolarising shifts. During this phase of activity the neurones were very strongly coupled
5. Thus barbiturates and volatile anaesthetics have markedly different mechanisms of action on electrically coupled neurons.

Bibliography

1. Richards C D. "On the mechanism of barbiturate anaesthesia". *The Journal of Physiology* 227.3 (1972): 749-767.
2. Richards C D. "Actions of general anaesthetics on synaptic transmission in the CNS". *British Journal of Anaesthesia* 55.3 (1978): 201-207.
3. Richards C D. "Actions of general anaesthetics on synaptic transmission". *British Journal of Anaesthesia* 55.3 (1983): 287-293.
4. Winlow W., *et al.* "Modulation of reconstructed peptidergic synapses and electrical synapses by general anaesthetics". *Toxicology Letters* 100.101 (1998): 77-84.
5. Halsey MJ., *et al*. "Potency and physical properties of inhalational anaesthetics". *General anaesthesia* (1989a): 7-18.

6. Halsey M J., *et al*. "Molecular mechanisms of anaesthesia". *General anaesthesia* (1989b): 19-29.
7. Daniell LC. "Effect of anaesthetic and convulsant barbiturates on N-Methyl-D-Aspartate receptor-mediated calcium flux in brain membrane vesicles". *Journal of Pharmacology* 49.5 (1994): 1-7.
8. Antkowiak B. "How do general anaesthetics work?" *Naturwissenschaften* 88.5 (2001): 201-213.
9. Girdlestone., *et al*. "The actions of halothane on spontaneous activity, action potential shape and synaptic connections of the giant serotonin-containing neuron of Lymnaea stagnalis (L.)". *Comparative Biochemistry and Physiology* 93.2 (1989): 333-339.
10. Walcourt-amakederemo A and Winlow W. "Halothane induces paroxysmal depolarisation shifts in synaptically isolated Lymnaea neurons in situ and in culture". *The Journal of Physiology* 473 (1993): 187.
11. Moghadam HF. "Effects of general anaesthetics and free radicals on cultured neurons Lymnaea stagnalis". (1996).
12. Woodall AJ., *et al*. "Anesthetic treatment blocks synaptogenesis but not neuronal regeneration of cultured Lymnaea neurons". *Journal of Neurophysiolog* 90.4 (2003): 2232-2239.
13. Winlow W., *et al*. Novel and orthodox responses of in vitro reconstructed synapses to volatile anaesthetics". *Progress in Anaesthetic Mechanisms* 6 (2000): 299-305.
14. Winlow., *et al*. "Modulation of reconstructed peptidergic synapses and electrical synapses by general anaesthetics". *Toxicology Letters* 100-101(1998): 77-84.
15. Spencer GE., *et al*. "Halothane affects both inhibitory and excitatory synaptic transmission at a single identified molluscan synapse, *in vivo* and *in vitro*". Brain Research 714.1-2 (1996): 38-48.
16. Spencer GE., *et al*. "Halothane-induced depression at both an in vivo and in vitro reconstructed synapse between neurons of Lymnaea". *Journal of Neurophysiology* 74.6 (1995): 2604-2613.
17. Onizuka S., *et al*. " Long-term Exposure to Local but Not Inhalation Anesthetics Affects Neurite Regeneration and Synapse Formation between Identified Lymnaea Neurons". *Anesthesiology* 102.2 (2005):353-363.
18. Onizuka S., *et al*. "Lidocaine treatment during synapse reformation periods permanently inhibits NGF-induced excitation in an identified reconstructed synapse of Lymnaea stagnalis". *Journal of Anesthesia* 26.1 (2012): 45-53.
19. Qazzaz M and Winlow W. "Effect of volatile anaesthetics on the electrical activity and the coupling coefficient of weakly electrically coupled neurons". *Acta Biologica Hungarica* 50.1-2 (1999): 199-213.
20. Qazzaz M and Winlow W. "Differential effects of volatile anaesthetics and barbiturates on electrical coupling between identified molluscan neurons". *The Journal of Physiology* 489 (1995): 21.
21. Qazzaz M and Winlow W. "Volatile anaesthetics diminish electrical coupling between identified molluscan neurons in the presence and the absence of met-enkephalin". *The Journal of Physiology* 497 (1995): 116-117.
22. Terrar DA and Victory JGG. "Influence of halothane on electrical coupling in cell pairs isolated from guinea pig ventricle". *British Journal of Pharmacology* 94.2 (1988): 509-514.
23. Burt JM and Spray DC. "Volatile anaesthetics block intracellular communication between neonatal rat myocardial cells". *Circulation Research* 65.3 (1989): 829-837.
24. Johnston MF., *et al*. "Interaction of anaesthetics with electrical synapses". *Nature* 286.5772 (1980): 498-500.
25. Wentlandt K., *et al*. "General Anesthetics Attenuate Gap Junction Coupling in P19 Cell Line". *Journal of Neuroscience Research* 81.5 (2005):746–752.
26. Wentlandt K., *et al*. "General Anesthetics Inhibit Gap Junction Communication in Cultured Organotypic Hippocampal Slices." *Anesthesia and Analgesia* 102.6 (2006):1692–1698.
27. Bennett MVL. "Gap junctions as electrical synapses". *Journal of Neurocytology* 26 (1997): 349-366.
28. Richards CD. "Anaesthetic modulation of synaptic transmission in the mammalian CNS". *British Journal of Anaesthesia*". 89.1 (2002): 79-90.
29. Pereda AE., *et al*. "Gap junction-mediated electrical transmission: regulatory mechanisms and plasticity". *Biochimica et Biophysica Acta* 1828.1 (2013): 134-146.

30. Mantz J., *et al*. "Effects of general anesthetics on intercellular communications mediated by gap junctions between astrocytes in primary culture". *Anesthesiology* 78.5 (1993): 892-901.
31. Dermietzel R., *et al*. "Gap junctions between cultured astrocytes: immunocytochemical, molecular, and electrophysiological analysis". *The Journal of Neuroscience* 11.5 (1991): 1421-1432.
32. Benjamin PR. and Winlow W. "The distribution of three wide acting synaptic inputs to identified neurons in the isolated brain of Lymnaea stagnalis". *Comparative Biochemistry Physiology* 70.3 (1981): 293-307.
33. Janse C., *et al*. "Age-related decrease in electrical coupling of two identified neurons in the mollusc Lymnaea stagnalis". *Brain Research* 376.1 (1986): 208-212.
34. Vlieger T., *et al*. "The role of membrane properties, synaptic input and electrical junction in determination of spike output of a pair of peptidergic neurons in the mollusc Lymnaea stagnalis". *Acta Biologica Hungarica* 43.1 (1992): 137-146.
35. Janse C., *et al*. "Central and peripheral neurons in oxygen perception in the pulmonate snail Lymnaea stagnalis". *Comparative Biochemistry and Physiology* 82.2 (1985): 459-469.
36. Loewenstein WR. "Junctional intracellular communication: the cell-to-cell membrane channel". *Physiological Reviews* 61.4 (1981): 829-913.
37. De Mello WC. "Cell-to-cell communication in the heart and other tissues". *Progress in Biophysics and Molecular Biology* 39 (1982): 147-182.
38. He D and Burt JM. "Mechanism and Selectivity of the Effects of Halothane on Gap Junction Channel Function". *Circulation Research* 86.11 (2000): 104-109.
39. Audesirk G., *et al*. "Variability and frequent failure of Lucifer yellow to pass between two electrically coupled neurons in Lymnaea stagnalis". *Journal of Neurobiology* 13.4 (1980): 369-375.
40. Soffe R. and Benjamin PR. "Morphology of two electrically coupled giant neuroscretory neurons in the snail Lymnaea stagnalis". *Comparative Biochemistry Physiology* 67.1 (1980): 35-46.
41. Bennett MV. "Physiology of electrotonic junctions". *Annals of the New York Academy of Sciences* 137.2 (1966): 509-539.
42. McCrohan CR and Benjamin PR. "Synaptic relationships of the cerebral giant cells with motoneurons in feeding system of Lymnaea stagnalis". *Journal of Experimental Biology* 85 (1980): 169-186.
43. De Mello WCI. "Effect of isoproterenol and 3-isobutyl-1-methylxanthine on junctional conductance in heart cell pairs." *Biochimica Biophysica Acta* 1012.3 (1989): 291-298.
44. Girdlestone D and Winlow W. "A relationship between neuronal topography and responses to anaesthetics in Lymnaea". *The Journal of Physiology* 386 (1987): 33.
45. Yar T. "The effects of halothane on cultured Lymnaea neurons". Ph.D. Thesis University of Leeds, (1992) UK.
46. Winlow W., *et al*. "Multiple cellular and subcellular actions of general anaesthetics on cultured molluscan neurons". *Acta Biologica Hungarica* 46 (1995): 381-393.
47. Spencer GE. "The effects of halothane on peptidergic responses of molluscan neurons". Ph.D. Thesis. University of Leeds, (1993) UK.
48. Davies DN., *et al.* "A comparison of arterial and arterialized venous concentrations of halothane". *British Journal of Anaesthesia* 44.6 (1972): 548-550.
49. Qazzaz M and Winlow W. "Volatile anaesthetics and barbiturates have different actions on the input resistance of an identified molluscan neuron". *British Journal of Anaesthesia* 75 (1995b): 656-657.
50. Franks NP and Lieb W R. "Selective effects of volatile general anaesthetics on identified neurons". *Annals of the New York Academy of Sciences* 625 (1991): 54-70.
51. Franks NP and Lieb WR. "Selective actions of volatile general anaesthetics at molecular and cellular levels." *British Journal of Anaesthesia* 71.1 (1993): 65-76.
52. Franks NP and Lieb WR. "Molecular and cellular mechanisms of general anaesthesia". *Nature* 367 (1994): 607-614.

53. Haydon PG., *et al*. "The effect of menthol on central neurons of the pond-snail Lymnaea stagnalis (L)". *Comparative Biochemistry and Physiology Part C: Comparative Pharmacology* 73.1 (1982): 95-100.
54. Spencer GE., *et al*. "Halothane-induced synaptic depression at both *in vivo* and *in vitro* reconstructed synapses between identified Lymnaea neurons". *Journal of Neurophysiology* 74.6 (1995): 2604-2613.
55. Spencer GE., et al. "Halothane affects both inhibitory and excitatory synaptic transmission at a single identified molluscan synapse, *in vivo* and *in vitro*". Brain Research 714.1-2 (1996): 38-48.
56. Zimmerman SA., et al. "Potentiation of γ-aminobutyric acid receptor Cl- current correlates with *in vivo* anaesthetic potency". *Journal of Pharmacology and Experimental Therapeutics* 270.3 (1994): 987-991.
57. Pauil E and El-Beheiry H. "Anaesthetic suppression of transmitter actions in neocortex". *British Journal of Pharmacology* 101.1 (1990): 61-66.
58. Lin L., et al. "Enlfurane inhibits NMDA, AMPA and Kainate-induced currents in Xenopus laevis oocytes expressing mouse and human brain mRNA". *Federation of American Societies for Experimental Biology Journal* 7.5 (1993): 479-485.
59. Gage PW and Robertson B. "Prolongation of inhibitory postsynaptic currents by pentobarbitone, halothane and ketamine in CA1 pyramidal cells in rat hippocampus". *British Journal of Pharmacology* 85.3 (1985): 675-681.
60. Arumugam H., *et al*. "Use of calcium imaging for analysis of neuronal gap junction coupling." *Neuroscience Letters* 445.1 (2008): 26-30.
61. Konopacki J., *et al*. "The electrical coupling and the hippocampal formation theta rhythm in rats". *Brain Research Bulletin* 107 (2014): 1-17.
62. Stavermann M., *et al*. "Calcium-induced calcium release and gap junctions mediate large-scale calcium waves on olfactory ensheathing cells in situ". *Cell Calcium* 58.2 (2015): 215-225.
63. Wojtczak J. "Electrical uncoupling induced by general anaesthetics: a calcium independent process? In Gap Junctions." *Cold Spring Harbor Laboratory* (1985): 167-177.
64. Niggli E., *et al*. "Effects of general anaesthetics on current flow across membranes in guinea pig myocytes". *American Journal of Physiology* 256.2 (1989): C273-C281.
65. Spray DC., *et al*. "Gating of gap junctional channels". *Biophysical Journal* 45.1 (1984): 219-230.
66. Perez-Valaquez JL., *et al*. "Modulation of gap junctional mechanisms during calcium-free induced field burst activity: possible role for electrotonic coupling in epileptogenesis". *Journal of Neuroscience* 14.7 (1994): 4308-4317.
67. Pérez-Armendariz EM., *et al*. "Characterisation of gap junction between pairs of Lydig cells from mouse testis". *American Journal of Physiology* 267.2 pt1 (1994): C570-C580.
68. Spray DC., *et al*. "Characterisation of gap junction as between cultured leptomeningeal cells". *Brain Research* 568 1-2 (1991): 1-14.
69. Qazzaz M and Winlow W. "Volatile anaesthetics decrease electrical coupling between identified molluscan neurons". *Proceeding of the Second Joint Meeting of Physiological Society of Japan UK and Eire* (1995d): 31.
70. De Mello WC. "Gap junctional communication in excitable tissues; the heart as a paradigm". *Progress in Biophysics and Molecular Biology* 61 (1994): 1-35.
71. Daniell LC and Harris RA. "Neuronal intracellular calcium concentrations are altered by anaesthetics: relationship to membrane fluidisation". *Pharmacology* 245.1 (1988): 1-7.
72. Ahmed IA and Winlow W. "Halothane raises intracellular calcium concentration ([Ca^{2+}]i) in cultured molluscan neurons". *Journal of Physiology* 483 (1995): 190P.
73. De Mello WC. "Increase in junctional conductance caused by Isoproteronol in heart cell pairs is suppressed by cAMP-dependent protein kinase inhibitor". *Biochemical and Biophysical Research Communications* 154.2 (1988): 509-514.
74. Juszczak GR and Swiergiel AH. "Properties of gap junction blockers and their behavioural, cognitive and electrophysiological effects: Animal and human studies". *Progress in Neuro-Psychopharmacology & Biological Psychiatry* 33.2 (2009) 181-198.

Volume 2 Issue 4 November 2015