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Essay Title: Role of Nitrogen Lipid Solubility in the Development of Inert Gas Narcosis in Diving

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Abstract

The growing popularity of SCUBA diving and use of divers in the oil and gas industries means a rising number of people are exposed to the dangers of underwater pressurised breathing equipment. These risks include decompression sickness, barotrauma, and other acute effects of breathing from a pressurised gas supply. One lesser understood risk of deep diving is the narcotic effects of inert gases, such as nitrogen, under high pressure. Breathing nitrogen-containing gas mixtures at depths exceeding 30msw (98ft) impedes the performance of physical and mental tasks, and at extreme depths (exceeding 90msw/295ft) can lead to loss of consciousness and drowning. The narcosis also impedes diver's ability to carry out rescue and other crucial skills, compromising their own and others safety. Currently, the mechanisms underlying inert gas narcosis are not well understood, but many theories may explain this phenomenon. These include physical disruption of the lipid membrane, interactions with a range of receptors, and synaptic interaction of hyperbaric inert gases. This article aims to explore one of the currently available theories for the mechanisms of inert gas narcosis: lipid bilayer disruption.

Keywords: Inert Gas Narcosis, Nitrogen, Diving, Lipid solubility

Abbreviation: atm, atmospheres of pressure; bar, barometric pressure (1bar = 1atm = 750.06mmHg); BSAC, British sub-aqua club; CCR, closed-circuit rebreather; CNS, central nervous system; DAN, Diver Alert Network; DPPC, dipalmitoylphosphatidylcholine ($C_{40}H_{80}NO_8P$); EAN, enriched air nitrox; GABA, Gamma-aminobutyric acid; HPNS, high pressure nervous syndrome; IGN, inert gas narcosis; MAC, minimum alveolar concentration; msw, metres of seawater (depth); SCUBA, self-contained underwater breathing apparatus;

Introduction

Nitrogen narcosis was first notably described in 1826 by a French physician, Colladon, who compared his mental state to having drunk alcohol. Colladon made this observation at a relatively shallow depth of 20m. (Rocco et al., 2019). However, it was not until 35 years later, in 1861, that the state of nitrogen narcosis was described by a professional diver, J. Green, following dives to depths exceeding 45m using air. Mr Green described his state as "feeling of excitement followed by drowsiness" and was the first person to identify the risk posed by this state to the divers' safety. (Unsworth, 1966)

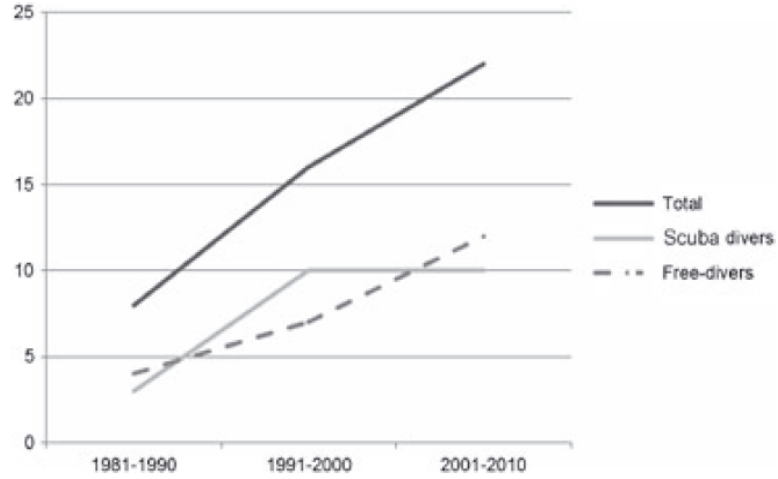

Figure 1 Number of diving related deaths during the three decades between 1981 and 2010 in Primorje-Gorski Kotar County in Croatia. Adapted from (Stemberga et al., 2013)

The risks of the IGN are now well understood and taught to almost all divers certified by a major organisation (such as PADI, BSAC, SDI, TDI). The significance of these risks is especially highlighted to divers training to dive to depths of 30msw or more. The signs and symptoms of the IGN get progressively more severe with increasing inspired nitrogen partial pressures, as seen in Table 1, and pose an increasing risk to the diver and their buddies. However, when the depth is decreased, most of the signs and symptoms are alleviated (except in extreme cases), though a time delay may be present between the depth reduction and alleviation of symptoms. The recovery time course has also been shown to be affected by the specific breathing mixture used by the diver. (Rocco et al., 2019). Rocco et al. showed that the divers using Trimix 21/35 (21% oxygen, 35% helium, and 44% nitrogen) took longer to

recover from the effects of IGN when compared to both air and heliox (21% oxygen, 79% helium, and 0% nitrogen). However, the exact time delay in recovery was not measured.

Furthermore, Rocco et al. noted that the recovery time could not be compared between the heliox group and other groups due to different decompression profiles. In their study, the air and trimix groups used EAN50 (50% oxygen and 50% nitrogen) for the ascent to decompression stops, whereas the heliox group continued using heliox from CCR. (Rocco et al., 2019)

Table 1 Typical clinical features of nitrogen narcosis with increasing partial pressures of nitrogen. The depth equivalent shows the depth at which the nitrogen partial pressure reaches the levels defined in the nitrogen partial pressure column, assuming a dive with air as breathing mixture (79% nitrogen, 21% oxygen). The signs and symptoms recorded during a dry dive in a compression chamber. Adapted from (Levett & Millar, 2008)

N2 Partial Pressure (bar)	Depth equivalent with air (msw/ft)	Signs and symptoms
2-4	15-40 (50-130)	Mild impairment of performance of unpractised tasks Mild euphoria
4	40 (130)	Impaired reasoning and immediate memory Delayed response to visual and auditory stimuli Increased reaction time
4-6	40-75 (130-245)	Overconfidence and fixed thinking Calculation errors
6	75 (245)	Impaired judgement Hallucinations
6-8	75-90 (245-295)	Laughter (approaching hysteria) or anxiety Talkative, occasional dizziness
8	90 (295)	Severely impaired intellectual performance Mental confusion, impaired concentration
10	120 (390)	Stupefaction
>10	>120 (390)	Hallucinations, unconsciousness, death

The alleviation of the symptoms with the decrease in depth makes it extremely hard to conclude whether IGN has contributed to a diver's fatality. The majority of the data about the fatalities related to IGN has been produced using interviews of the buddies and the dive profile reviews. (Busuttil & Obafunwa, 1995; Caruso, 2011) However, this is an unreliable method of data collection. As seen in Table 2, as many as 64% of the divers who died during diving related activities between 1972 and 2006, did not have a buddy nearby. A further 18% were separated from the buddy during the accident. The measurement of the impact of IGN is further complicated by the lack of tracking of non-fatal incidents potentially caused by IGN.

Table 2 Buddy status prior and during the fatal accidents (n=288). Data collected from National Coronial Information System in Australia. Adapted from (Lippmann, 2011)

Buddy Status	Occurrence (%)
Separated Before	49
With Buddy	18
Separated during	17
Solo	16

The latest report published by BSAC in 2019, has identified 3 incidents that were caused by IGN. These incidents equate to 0.8 per cent of the incidents described in the report. (BSAC., 2019). The number of fatal incidents identified to have been caused by the IGN is unknown due to the limitations of investigations described above. However, in 2010, the number of fatal incidents caused by IGN in the UK was estimated to be 3.6% of fatal incidents reported to DAN. However, IGN is a likely contributor to a much greater number of incidents. (Vann & Lang, 2011)

Despite the scientific and diving communities knowing about the effects and dangers of IGN for over 150 years, the underlying mechanisms of the IGN are poorly understood. Multiple theories attempt to explain the phenomenon of IGN including lipid solubility, co-agonistic interaction of inert gas with GABA receptors, and physical disturbance of lipid bilayers. This article aims to explore the lipid bilayer disruption theories of IGN.

Discussion

Multiple evidence-supported theories may explain the development of IGN. Nearly all of these hypotheses are shared with those used to explain the action of inhalation general anaesthetics. One of the few things known about narcosis's mechanism is that the primary site of action in the brain is the pre- and post-synaptic regions.

Meyer-Overton Hypothesis

One of the commonly quoted explanations for the IGN is the structural changes of the neurones' lipid bilayer membranes in the CNS. (Antkowiak, 2001; Levett & Millar, 2008; Miller et al., 1973). This theory of the anaesthetic gas mechanism of action was first suggested by Meyer and Overton in 1899 and 1901, respectively (Meyer, 1899; Overton, 1901). Meyer and Overton have described the correlation seen in Figure 2, where the anaesthetic potency is directly proportional to the gas's solubility in lipids (measured as the oil : gas partition coefficient) (Antkowiak, 2001). This data is further supported by the data presented in Table 3, which shows the relative narcotic potency and lipid solubilities of common inert gases. The anaesthetic potency is usually measured as the inverse of the minimum alveolar concentration (MAC).

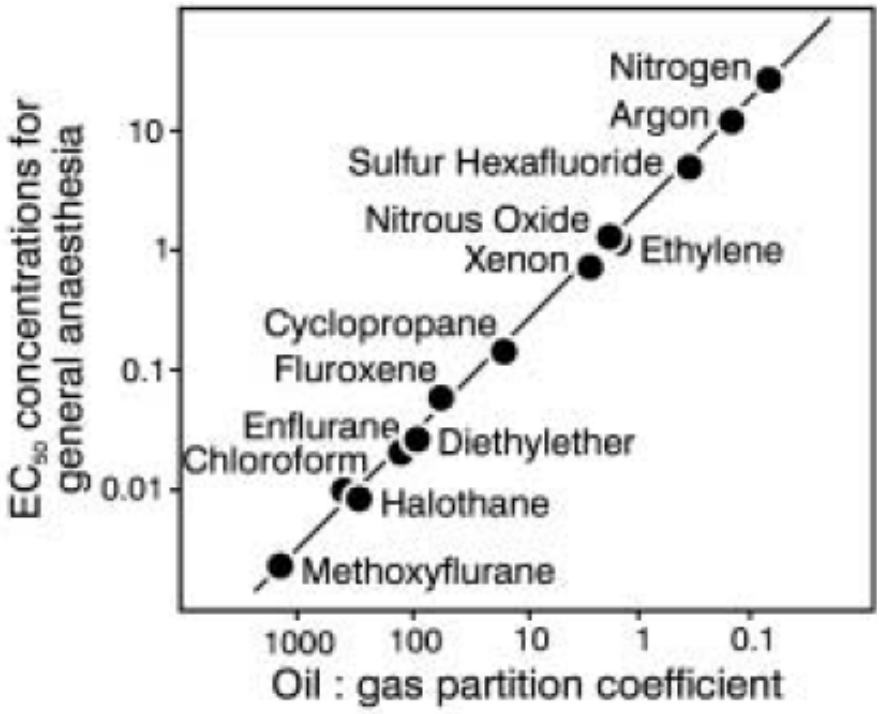

Figure 2 The correlation between the oil : gas partition coefficient and the EC50 concentration for general anaesthetics as well as Xenon and Nitrogen gases. The EC50 for the gases was measured as gas concentration required to prevent nocifensive reflexes in 50% of animals tested. Adapted from (Antkowiak, 2001)

Interestingly, several general anaesthetics – including halothane and methoxyflurane – discovered after Meyer and Overton published their findings also fit this correlation (Antkowiak, 2001; Bergadano, Lauber, Zbinden, Schatzmann, & Moens, 2003). The development of IGN with nitrogen has been first attributed to the oil : gas partition in 1935 by Behnke, Thompson, and Motley (Behnke, Thomson, & Motley, 1935). Behnke et al. have found that if nitrogen was replaced by helium in the breathing mixture, in effect using trimix or heliox mixtures, the severity of the nitrogen narcosis could be reduced or abolished entirely. (Behnke et al., 1935; Unsworth, 1966). However, Behnke's study is limited since the effects of the narcosis were measured subjectively depending on the difficulty of performing a practised task.

Table 3 Comparison of common gases lipid solubilities and relative potencies. The relative potencies are relative to nitrogen (nitrogen relative potency = 1). (Lawrence, Loomis, Tobias, & Turpin, 1946; Rostain & Lavoute, 2016; Unsworth, 1966)

Gas	Lipid Solubility (mg/ml)	Relative Potency
Helium (He)	0.015	0.2
Hydrogen (H_2)	0.036	0.6
Nitrogen (N_2)	0.067	1.0
Oxygen (O_2)	0.11	1.7
Argon (Ar)	0.14	2.3
Carbon Dioxide (CO_2)	1.34	20.0
Xeon (Xe)	1.7	25.6

The lipid solubility hypothesis has two explanations for the mechanism of narcosis.

Lipid Bilayer Expansion Hypothesis

The first theory is known as the lipid biolayer expansion or critical volume hypothesis. It states that the lipophilic gas diffuses and accumulates in the hydrophobic regions of the lipid bilayer of the neurones in the CNS. The accumulation of the gas in the membrane causes physical distortion of the lipid membrane, thus producing an expansion of the hydrophobic site. This expansion of the hydrophobic site was thought to interfere with the functioning of the ion channels in neurones, producing the narcotic effect. This mechanism was first proposed by Miller and Smith in 1973. During their study, Miller and Smith used 10-12 Sprague-Dawley rats to study whether the effects of anaesthesia using 4 anaesthetics (methohexitone, thiopentone, propanidid, and ketamine) could be reversed using high pressure. Miller and Smith have found that the dose of the anaesthetic required to maintain anaesthesia in the rats increased with increased gas pressure (Miller and Smith used up to 100 atm of helium with a 0.6 atm of oxygen), as seen in Figure 3 and Figure 4. Miller and

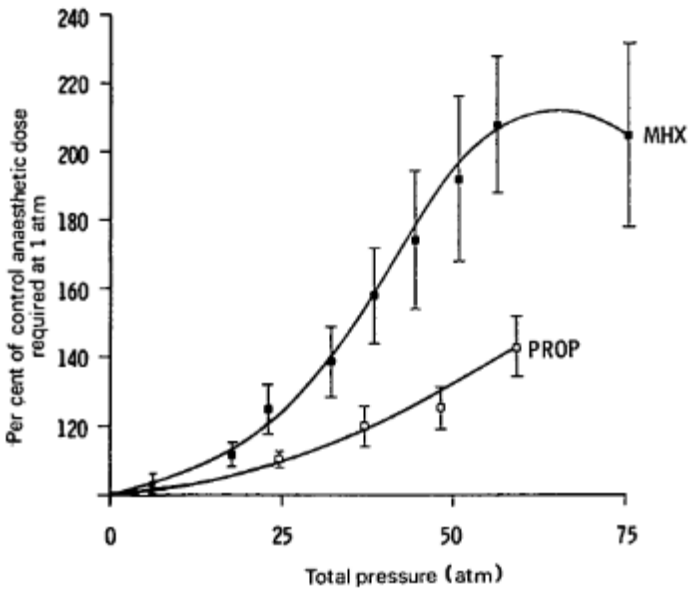

Figure 3 Increase in anaesthetic dose at pressure for agents potentiating convulsions – Methohexitone (MHX) and Propanidid (PROP). The data is presented as mean ± SEM for the animals in "good physiological state". (Miller et al., 1973)

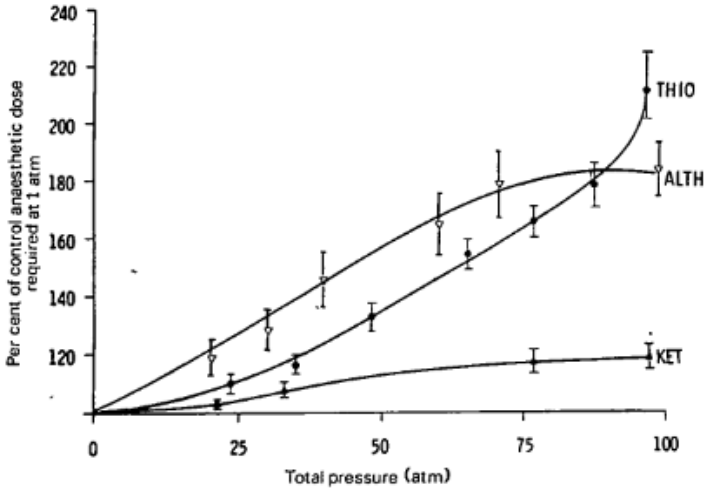

Figure 4 Increase in anaesthetic dose at pressure for the agents which could be studied up to 100 atm without significant changes in physiological state - Thiopentone (THIO) and Ketamine (KET). Data for Althesin (ALTH) was taken from Bailey et al. Data presented as mean ± SEM. (Bailey, Green, Halsey, & Wardley-Smith, 1972; Miller, Paton, Smith, & Smith, 1973)

Smith suggested that the increased pressure would reduce the extent of the hydrophobic site, reducing the potency of the anaesthetic. (Miller et al., 1973)

Miller had to terminate the experiments with methohexitone and propanidid due to the animals experiencing convulsions, likely due to the high-pressure nervous syndrome (HPNS) caused by extreme partial pressures of helium. (Cromer, Bennett, Hunter, & Zinn, 1979; Miller et al., 1973)

This theory, however, has some limitations, such as:

1. Absence of anaesthesia produced by temperature changes (Franks & Lieb, 1982)
2. Variation in potencies between stereoisomers of the drug (Weiskopf, Nau, & Strichartz, 2002)
3. Existence of nonimmobilizers (e.g. helium) (Koblin et al., 1998)

Due to the limitations of the lipid bilayer expansion hypothesis, an updated hypothesis has been suggested, known as membrane lateral pressures hypothesis.

Membrane Lateral Pressure Hypothesis

The membrane lateral pressure hypothesis was proposed by Cantor in 1998 (Cantor, 1998). The cell membrane's lipid bilayer experiences a lateral pressure (also described as local transverse pressure). The lateral pressure in the bilayer is non-uniform, and thus, sections of the membrane have distinct pressure profiles (Safran, 1995; Urban, 2002). Cantor suggested that the dissolving of the anaesthetic or inert gas in the membrane may induce changes in the local pressure profiles. He also noted that, despite the relative changes in the pressure being small, the changes would be "large in absolute magnitude since the pressures themselves are enormous" (Cantor, 1998).

However, the mechanistic implications of these changes in the lateral pressures are not understood. Cantor proposed that the opening of the ion channel would lead to non-uniform changes in the cross-sectional area of the ion channel protein. The ion channel in an open state experiences a larger increase in the local lateral pressure around the aqueous interface due to a more significant increase in the protein's cross-sectional area at the interface. The part of the ion channel protein in the middle of the bilayer experiences a smaller increase or even a decrease in the cross-sectional area. Thus the changes in the local lateral pressure would be less. (Cantor, 1998)

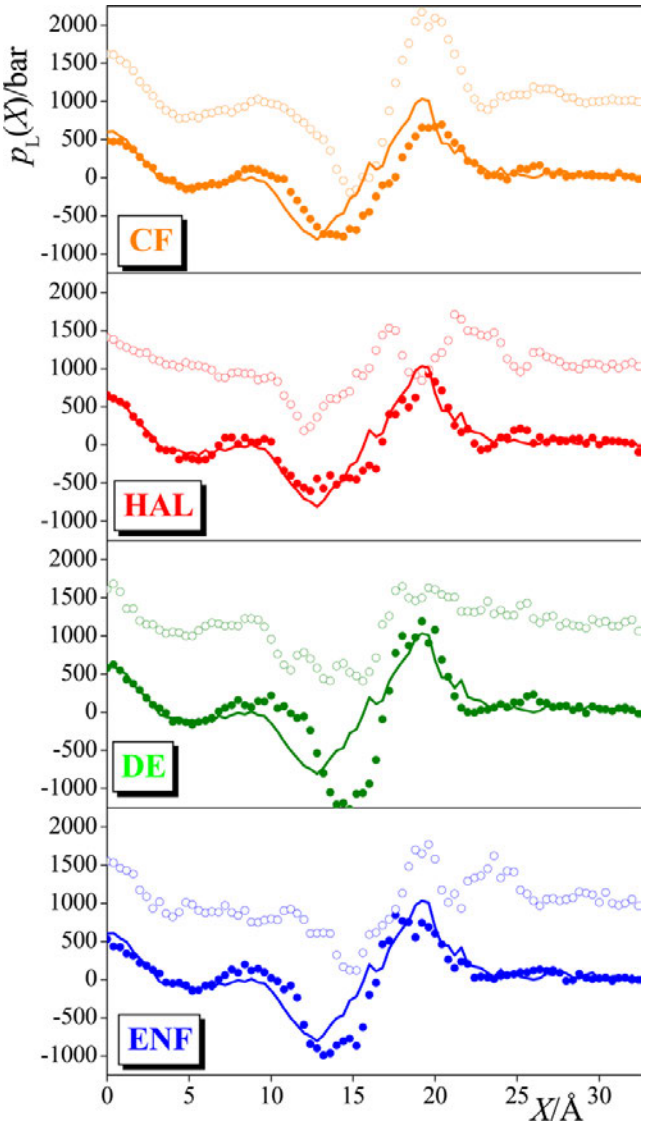

Figure 5 Comparison of the lateral pressure profile across the pure DPPC lipid membrane at 1 bar (solid curves) with the membranes containing anaesthetics, simulated at 1 bar (filled circles) and 1000 bar (open circles).

Top panel: membrane containing chloroform (CF); second panel: membrane containing halothane (HAL); third panel: membrane containing diethyl ester (DE); bottom panel: membrane containing enflurane (ENF). Data was obtained using computer simulation. X = 0 Å corresponds to the middle of the bilayer. Error bars are always smaller than the symbols. Adapted from (Fábián, Sega, Voloshin, Medvedev, & Jedlovszky, 2017)

The presence of an anaesthetic or inert gas in the middle of the lipid bilayer would alter the local lateral pressures and thus alter the thermodynamic equilibrium of the closed and opened states of the ion channels towards the closed state. The change in the equilibrium would lead to an increase in the energy required for the opening of the ion channel, thus decreasing the likelihood that the ion channel would open. (Cantor, 1998; Gruner & Shyamsunder, 1991)

The lateral pressure hypothesis is supported by the evidence recently obtained by Fabian et al., who used a computer simulation to show the lateral pressure profiles in the DPPC bilayer membrane. The results obtained by Fabian et al. are presented in Figure 5. The computer simulation was run to describe the lateral pressure profiles throughout a DPPC lipid membrane at 1atm without anaesthetic, 1atm with 4 different anaesthetics, and 1000atm with the 4 anaesthetics. The four anaesthetics simulated were chloroform, halothane, diethyl ester, and enflurane. The results obtained by Fabian et al. show that the anaesthetics had significantly reduced the lateral pressure in the lipid chain ester groups region (X = 12-13Å). The change in the lateral pressure profile was largely reversed by the global pressure increase to 1000atm. (Fábián et al., 2017)

Lipid Bilayer Disruption Limitations

However, the lipid bilayer disruption hypotheses are limited due to several exceptions and limitations. An example of this is a finding by Simon et al. from 1979. The halothane potency increased with a reduction in temperature, despite the bilayer-saline partition coefficient of halothane increasing with increasing temperature. (Simon, MCintosh, Bennett, & Shrivastav, 1979)

This limitation is overcome by the hypothesis that the inert gases and general anaesthetics work by acting on the $GABA_A$ receptors in the brain and potentiate the action of endogenous GABA to produce narcotic and anaesthetic effects. The $GABA_A$ receptors are ligand gated ion channels that are selectively permeable to chloride ions. The opening of these ion channels, following activation by GABA, allows an influx of chloride ions into the neurone. The negatively charged chloride ions hyperpolarise the postsynaptic neurone and decrease the chance of a successful action potential firing. Thus, the $GABA_A$ receptor is used as part of inhibitory pathways.(Abraini, Kriem, Balon, Rostain, & Risso, 2003;

Antkowiak, 2001) The $GABA_A$ receptors also contain allosteric binding sites for molecules that modulate its activity. It is thought that several general anaesthetics act by binding to these allosteric sites and potentiate the action of the GABA. Research carried out by Abraini et al. has found that $GABA_A$ selective antagonists can be used to counter the narcotic effects of hyperbaric nitrogen, suggesting that nitrogen may act on the $GABA_A$ receptors in a similar manner to the general anaesthetics (Abraini et al., 2003).

Concluding Remarks

In conclusion, the mechanism of inert gas narcosis is likely to be multifactorial involving interaction of the lipid disruption theories described in this essay and the effect of inert gas on the GABA and/or $GABA_A$ receptors in the brain. The future research should focus on further exploring the interaction of hyperbaric nitrogen with lipid membranes in vitro and in vivo, instead of computer and mathematical simulations used in previous research. The improved understanding of the mechanisms of general anaesthetics and inert gas narcosis will have a two-part effect. Firstly, it may improve the safety of SCUBA diving by potentially providing methods to overcome or limit the effects of narcosis. Secondly, it may uncover other drugs that may be used as general anaesthetics or sedatives, as well as improve safety of currently used medication.

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