Pupillometry is not sensitive to gas narcosis in divers breathing hyperbaric air or normobaric nitrous oxide

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Key words
Diving research; Nitrogen; Nitrous oxide; Physiology

Abstract
(Vrijdag XCE, van Waart H, Sleigh JW, Mitchell SJ. Pupillometry is not sensitive to gas narcosis in divers breathing hyperbaric air or normobaric nitrous oxide. Diving and Hyperbaric Medicine. 2020 June 30;50(2):115–120. doi: 10.28920/dhm50.2.115-120. PMID: 32557412.)
Introduction: Gas narcosis impairs divers when diving deeper. Pupillometry is sensitive to alcohol intoxication and it has been used in anaesthesia to assess nitrous oxide narcosis. It is a potential novel method to quantify narcosis in diving. The aim of this study was to evaluate pupillometry for objective measurement of narcosis during exposure to hyperbaric air or nitrous oxide.
Method: Pupil size in 16 subjects was recorded directly at surface pressure and during air breathing at 608 kPa (equivalent to 50 metres' seawater depth) in a hyperbaric chamber. Another 12 subjects were exposed to nitrous oxide at end-tidal percentages of 20, 30 and 40% in random order at surface pressure. Pupil size and pupil light reflex were recorded at baseline and at each level of nitrous oxide exposure.
Results: Pupil size did not significantly change during exposure to hyperbaric air or nitrous oxide. The pupil light reflex, evaluated using percentage constriction and minimum diameter after exposure to a light stimulus, was affected significantly only during the highest nitrous oxide exposure – an end-tidal level of 40%.
Conclusion: Pupillometry is insensitive to the narcotic effect of air at 608 kPa in the dry hyperbaric environment and to the effects of low dose nitrous oxide. Pupillometry is not suitable as a monitoring method for gas narcosis in diving.

Introduction

Divers venture underwater either for work or pleasure, and may experience nitrogen narcosis. The onset of narcosis symptoms is expected around 30 metres' sea water (msw) (4 atmospheres absolute pressure [atm abs], 405 kPa), when breathing air.[1] These symptoms influence the diver's capacity to make decisions and are a contributing factor in incidents.[2] Besides incidents, the reduced mental capacity affects the quality of work underwater, causing divers to make more mistakes or taking longer to complete their task.[3] Quantifying nitrogen narcosis has been investigated since Behnke first attributed the narcotic effects experienced during hyperbaric exposures to nitrogen.[4] Ideally narcosis could be continuously monitored in real time without interfering with diving activities.[5] Pupillometry is a potentially novel method to achieve this.

Pupillometry is the measurement of the pupil size and pupillary reflexes. The pupil size is subject to reflex responses to light/dark or pain stimuli. Pupil size and reflexes can be measured objectively with a portable infrared pupillometer with a light flash, which can calculate multiple variables such as latency of onset, magnitude of constriction and constriction velocities (Figure 1). Both the resting pupil size and light reflex are modulated by a feedback loop between the eye and the oculomotor nucleus in the pretectal area of the midbrain. Other areas in the brain influence this nucleus to dilate or contract the pupil based on general sympathetic and parasympathetic pathways.[6]

Previous research has shown that pupillometry can be used to screen workers for the influence of alcohol.[7] An increase in pupil diameter was measured directly after 0.6 $g \cdot kg^{-1}$ ethanol intake. The pupil light reflex has been used to quantify cognitive processes like attention, decision making and emotional arousal.[8–12] It has also been used to measure the effects of anaesthetic agents, including nitrous oxide,[6,13] and on the intensive care unit to monitor critically-ill patients.[14]

The behavioural manifestations of alcohol intoxication and nitrogen narcosis are quite similar.[15] Pupillometry has, to our knowledge, never been used to assess the narcotic effects of nitrogen in hyperbaric air breathing.

Figure 1
Metrics of the pupil light reflex. Maximum dilation (max) is the maximal pupil diameter before the light flash. Minimum dilation (min) is the minimal pupil diameter after the light flash. Latency is the time between the onset of the light flash and the onset of the pupil contraction. Constriction is calculated as (max-min)/max

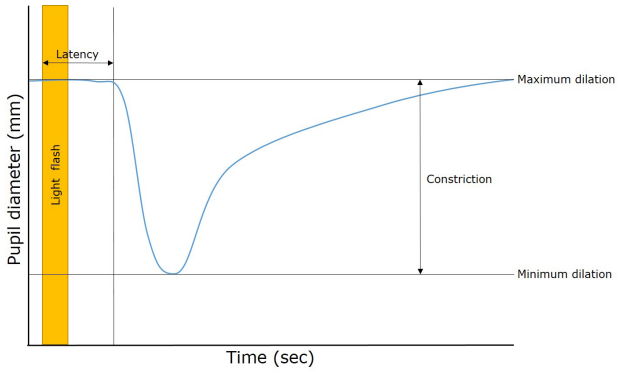
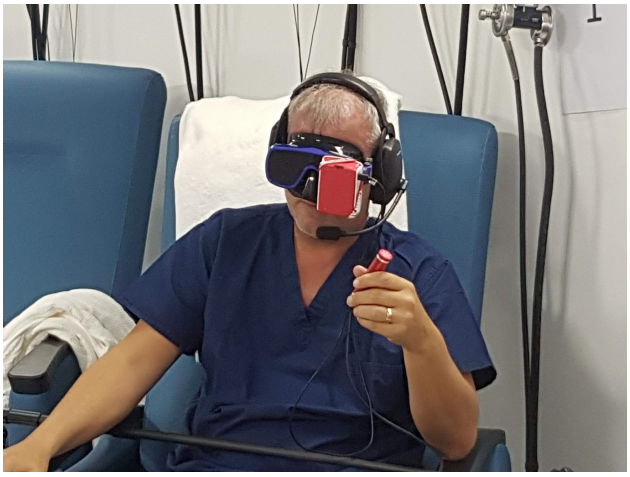


Figure 2
Participant wearing the pupillometry headset, while seated inside the hyperbaric chamber



Conducting narcosis experiments either during dives or inside a hyperbaric chamber are costly, labour and time intensive because of the incurred decompression and personnel involved to conduct hyperbaric experiments. In previous diving medical research, nitrous oxide has been used as a substitute to nitrogen narcosis.[16–19] Low dose nitrous oxide and hyperbaric nitrogen result in similar behavioural impairment measured with a range of psychometric tests.

The aim of this study was to evaluate the use of pupillometry for objective measurement of mild to moderate narcosis experienced during exposure to graded doses of nitrous oxide, and to hyperbaric air breathed at a pressure chosen to meet or exceed the maximum recommended depth for use of air during diving.

Methods

The study consisted of two experiments: pupillometry during air breathing at hyperbaric pressure; and during sea-level exposure to low-dose nitrous oxide.

TRIAL DESIGN AND PARTICIPANTS

The hyperbaric trial took place at the hyperbaric facility at Deep Dive Dubai, in March 2018. The study protocol was approved by the Dubai Scientific Research Ethics Committee of the Dubai Health Authority, United Arab Emirates (reference 10/2017_06).

The randomised, single-blind, cross-over, nitrous oxide trial took place at the Waikato Clinical School, University of Auckland, in July–August 2018. This study protocol was approved by the Health and Disability Ethics Committee, Auckland, New Zealand (reference 16/NTA/93) and was registered with the Australian New Zealand Clinical Trial Registry (ANZCTR) with Universal Trial Number U1111-1181-9722. These pupillometry measurements were a sub-study in a larger body of work investigating gas-induced narcosis that will be reported elsewhere.

Participants were eligible if they were certified, healthy adult divers, aged between 18 and 60 years and had normal visual acuity, either corrected or uncorrected. Participants were excluded if they were using recreational drugs, tobacco, psychoactive medication, excessive alcohol (> 21 standard drinks per week) or over five caffeine-containing beverages a day. All participants provided written informed consent. Participants abstained from any caffeinated drink on the measurement day, and from alcohol for at least 24 hours before the measurement. Participants had at least 6 hours of sleep the night before the measurement.

EQUIPMENT

A pupillometry device suitable for hyperbaric environments was built using an infra-red camera (PiNoir Camera V2, RS-components, the Netherlands) attached to a Raspberry Pi, a small computer (Raspberry Pi 3 Model B, RS-components, the Netherlands) mounted to a blacked-out scuba diving mask (Figure 2). The Pi was controlled by a web interface on a mobile device outside the hyperbaric chamber via a WiFi connection. The device took a picture of the eye, and the images were stored on a micro-SD card for off-line analysis. Unlike the proprietary pupillometer described below, the device built for the hyperbaric chamber did not measure pupillary reflexes in response to a light flash.

The pupil and iris diameter were determined from the stored images using Photoshop (Adobe, San Jose, CA, USA). Since distance between the camera and eye varied, the pupil diameter was corrected by using the fixed iris diameter, by calculating the ratio between them.

During the nitrous oxide experiments, a purpose-built PLR-200 Pupillometer (Neuroptics, Laguna Hills, CA,

Table 1
Demographic data for study subjects

Characteristic	Hyperbaric experiment *n* = 16	Nitrous oxide experiment *n* = 12
	Mean (range)	Mean (range)
Age (years)	35.3 (20–54)	36.25 (23–55)
Body mass index	23.8 (19.1–29.3)	26.3 (22.7–31.3)
Diving experience (years)	11.4 (1–29)	12.0 (1–36)
Dives	2,679 (15–10,000)	557 (20–1,500)
	n (%)	*n (%)*
Gender (male)	10 (62.5)	8 (66.7)
Certification:		
- Supervised diver	2 (12.5)	0
- Autonomous diver	2 (12.5)	6 (50.0)
- Dive Leader	1 (6.3)	0
- Dive instructor	11 (68.8)	6 (50.0)
- Technical diver	11 (68.8)	4 (33.3)
Experience (> 50 msw air)	8 (50.0)	7 (58.3)

USA) was used to record not just pupil diameter, but also pupillary reflexes in response to a 180 microwatt light flash administered for 154 milliseconds. Analysis of the recording was performed by the device to calculate the metrics mentioned below and stored for statistical analysis.

HYPERBARIC EXPERIMENTAL PROCEDURE

Sixteen divers volunteered to participate in this study. The chamber was a multi-place (10-person) rectangular hyperbaric chamber (Oxyheal 5000, National City, CA, USA). The lights inside the chamber were dimmed to 50% to minimise influence of ambient light on the pupillometry recording. Pupil size was then recorded inside the hyperbaric chamber at surface pressure immediately before compression. Participants were then compressed in groups of 2–4 persons to 608 kPa (equivalent to 50 msw depth) breathing environmental air. Upon arrival an acclimatisation period of five minutes was allowed to ensure onset of nitrogen narcosis before the recordings started. The infra-red photo of the eye was directly assessed for clarity and focus and if needed repeated to obtain one high quality image with the iris and pupil completely visible (not obscured by eye lids). After the measurements were finished, decompression was according to the US Navy decompression tables, including 100% oxygen breathing from 190 kPa to surface pressure.

NITROUS OXIDE EXPERIMENTAL PROCEDURE

Twelve divers volunteered to participate in this study. The experiment was conducted in a normobaric laboratory environment using a closed-circuit anaesthesia breathing loop (Vital Signs, Mexico) attached to an anaesthesia machine (S/5 Aespire, Datex-Ohmeda, Madison WI, USA). A normal scuba mouthpiece and a disposable anaesthetic antibacterial filter (Ultipor 25, Pall, Port Washington, NY, USA) constituted the interface with the participant. These two pieces of the breathing circuit were replaced for each participant which allowed use of the same breathing loop for multiple subjects. The nose was occluded with a nose clip. A gas sample line connected the mouthpiece filter to the anaesthetic monitor (GE Healthcare, Chicago, IL, USA) which measured the inspired fraction of oxygen, end-tidal pressure of carbon dioxide and end-tidal percentage of nitrous oxide breath by breath in real time.

The flow of nitrous oxide into the breathing circuit was titrated to maintain the desired end tidal percentage (20, 30 or 40%) of nitrous oxide. The remainder of the breathing gas was oxygen, with a continuous flow into the anaesthetic circuit of 2 $L \cdot min^{-1}$.

A baseline pupillometry measurement, before breathing nitrous oxide, was recorded while the participant was breathing 50% oxygen (balance nitrogen) on the circuit. Subjects then breathed nitrous oxide with an end-tidal level of 20, 30 and 40% in randomised order with a rest period of twenty minutes in between. The participants were blinded to the dose of nitrous oxide being administered. After 3–5 minutes of washing-in nitrous oxide at each dose, the device was placed in front of the right eye, covering the eye. While the camera was focussed on the pupil, the device applied one light flash to obtain the pupil light reflex. The measurement was repeated if, during the recording, the view of the pupil

Table 2
Pupillometry parameters in the nitrous oxide (N_2O) experiment (n = 12). Data are mean (SD)

Pupillometry parameter	Baseline	20% N_2O	30% N_2O	40% N_2O
Maximum dilation (mm)	5.5 (0.8)	5.4 (0.7)	5.4 (0.8)	5.6 (0.8)
Minimum dilation (mm)	3.9 (0.7)	3.9 (0.7)	4.0 (0.6)	4.2 (0.8)
Contraction (%)	-28.6 (5.6)	-27.8 (5.8)	-26.8 (3.8)	-24.8 (4.2)
Latency (ms)	237.5 (34.9)	237.5 (23.8)	239.2 (36.8)	225.8 (55.8)

Table 3
Mean differences with 95% confidence intervals (95% CI) in pupillometry parameters compared to baseline in pupillometry in the nitrous oxide (N_2O) experiment (n = 12). * indicates significant difference: $P < 0.05$

Pupillometry parameter	20% N_2O	30% N_2O	40% N_2O
Maximum dilation (mm)	0.1 (-0.2 to 0.4)	0.0 (-0.2 to 0.3)	-0.1 (-0.4 to 0.2)
Minimum dilation (mm)	0.0 (-0.3 to 0.3)	-0.1 (-0.3 to 0.2)	-0.3 (-0.6 to 0.0)*
Contraction (%)	-0.8 (-3.6 to 1.9)	-1.8 (-4.5 to 0.8)	-3.8 (-6.1 to -1.4)*
Latency (ms)	0.0 (-21.5 to 21.5)	-1.7 (-28.5 to 25.1)	11.7 (-29.7 to 53.0)

was blocked by a blink causing an inability to calculate the parameters. At the end of each exposure the nitrous oxide was washed-out of the anaesthetic circuit using oxygen with a fresh gas flow of 6 $L \cdot min^{-1}$.

OUTCOMES

The primary outcome was the absolute change in pupil diameter during exposure to nitrogen or nitrous oxide relative to baseline.[20] A secondary outcome of the nitrous oxide experiments was the pupillary light reflex, quantified by the percentage constriction (defined as maximum diameter (MAX) minus the minimum diameter (MIN) divided by MAX), and the latency (defined as the time between the light flash and the onset of the constriction) (Figure 1).

STATISTICAL ANALYSIS

Descriptive statistics were generated to characterise the study participants. All outcome measures were tested for normality and subsequently characterised by their mean and standard deviation (SD). Differences between baseline and intervention measures were analysed with paired t-tests and reported as mean difference with 95% confidence intervals. All data were analysed with SPSS version 25 (IBM, Armonk, NY, USA). Statistical significance was set at $P < 0.05$.

Results

HYPERBARIC NITROGEN EXPERIMENT

The 16 participants had between 15 and 10,000 dives, 11 were instructors or above, 11 were technical divers and half of the group had previous experience breathing air at 608 kPa or deeper (Table 1). The mean pupil-iris ratio did not change significantly while breathing air at 608 kPa (0.50 [SD 0.08]) compared to surface pressure baseline (0.51 [0.10]).

NITROUS OXIDE EXPERIMENT

The 12 participants had between 20 and 1500 dives, six were instructors or above, 11 were technical divers and seven had previous experience breathing air at 608 kPa or deeper (Table 1). The maximum pupil diameter (i.e., the resting state) and the latency did not change significantly during exposure to nitrous oxide at an end-tidal percentage of either 20, 30 or 40% compared to baseline (Table 2). However, the minimum diameter was significantly larger at the 40% nitrous oxide level (P = 0.047) compared to baseline with a mean difference of -0.3 mm, but not at lower concentrations. Pupil constriction was inhibited by nitrous oxide. This was only significant when baseline (-28.6%) was compared to nitrous oxide at an end tidal fraction of 40% (-24.8%) (P = 0.004) (Table 3).

Discussion

In this study pupillometry was insensitive to the narcotic effects of nitrogen in air breathed at 608 kPa, and low-dose nitrous oxide had only very small effects. Only during exposure to nitrous oxide at the end tidal = 40% level was there a small effect on the pupil reflex after a light flash. Since previous studies have clearly shown that similar nitrous oxide and hyperbaric air exposures cause significantly decreased cognitive performance[21] we conclude that pupillometry is unsuitable for monitoring the effects of this potentially dangerous degree of gas narcosis in divers.

Based on the effect previously reported in an alcohol study[7] we hypothesised that pupil diameter would be affected by

the exposure to air at 608 kPa. The doses used in the alcohol study (0.3 and 0.6 g·kg^{-1}) were approximately equivalent to 2 and 4 standard drinks for a 70 kg male and are in the same range as another alcohol study, in which oral alcohol (0.5 g·kg^{-1}) produced a similar reduction in cognitive performance as air dives to 40–45 msw.[15] Both alcohol and nitrogen have an impact on a wide variety of neural systems, and produce some similar effects. However, the lack of effect found with pupillometry in the present study using air breathing at 608 kPa, might indicate that alcohol and nitrogen influence the pupil feedback loop in the midbrain differently. A similar difference has been described, where the volatile anaesthetic agent ether affects pupil dilation, while other anaesthetic agents do not.[6]

The pupillary light reflex was slightly depressed when participants were exposed to sufficient inspired nitrous oxide to maintain an end tidal percentage of 40%. Others reported a similar result during general anaesthesia incorporating 60% nitrous oxide.[13] However, in the present study no light reflex depression was found at lower doses of nitrous oxide. Compared to air breathed at 608 kPa, an end tidal fraction of 40% nitrous oxide results in greater cognitive impairment.[18] This suggests that pupillometry is only sensitive to levels of impairment that exceed those likely to be produced in the air diving operational range of interest (surface to 50 msw; 101–608 kPa).

In addition, the small effect found at an end tidal nitrous oxide fraction of 40% was reliant on a light flash to measure the effect on the pupillary reflex. The need to expose the diver regularly to a light flash in order to assess the narcosis level, would also make it less suitable for continuous monitoring in a dive environment.

This study had a number of strengths, including exposure of subjects to both air at 608 kPa and nitrous oxide at surface pressure, the use of multiple concentrations of nitrous oxide to evaluate pupillometry response in a graded narcotic dose-response, and the use of two different pupillometry devices that showed similar results.

Some limitations need to be acknowledged. During the hyperbaric experiment only pupil dilation could be measured. Due to different face shapes, some divers had to push the mask to their face in order to get the camera in front of the eye causing some variation in the distance between eye and camera. We compensated for this, by measuring the iris diameter and calculating the ratio between the two of them. Narcosis is hard to measure objectively and accurately. In this trial there was no method available to monitor the narcotic effects systematically, and therefore it could be argued that perhaps these participants did not experience any narcosis However, impairment caused by breathing air at 608 kPa was assumed, given the extensive literature on nitrogen narcosis at this depth.[2,22] Similarly, we expected the nitrous oxide to have a cognitive impairment based on literature.[17] This was supported by informal observations of change in behaviour in all participants. Finally, we did not evaluate pupillometry in actual diving where other influences (such as hypercapnia) may enhance gas narcosis at any given depth. It is conceivable that pupillometry might be more sensitive under these circumstances.

Conclusion

In conclusion, pupillometry is insensitive to nitrogen narcosis in the operational depth range of interest (surface to 50 msw) and to the narcotic effects of low dose nitrous oxide. Hence, this method is not suitable as a monitoring method for gas narcosis in diving.

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Acknowledgements

We are grateful to all divers that participated in this study. Furthermore, we would like to acknowledge the staff of Deep Dive Dubai and the department of Anaesthesia at the Waikato Clinical School for their support during the data collection.

Conflicts of interest and funding

Professor Mitchell is the Editor of *Diving and Hyperbaric Medicine* Journal and had no role in managing the review process or the decision to accept this manuscript. These matters were managed by the European (deputy) Editor, Dr Lesley Blogg. There were no other conflicts of interest.

The nitrous oxide study was supported by funding from the Office for Naval Research Global (ONRG), United States Navy (N62909-18-1-2007).

Submitted: 18 September 2019
Accepted after revision: 08 December 2019