

Objective vs. Subjective Evaluation of Cognitive Performance During 0.4-MPa Dives Breathing Air or Nitrox

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- BACKGROUND:** Divers try to limit risks associated with their sport, for instance by breathing enriched air nitrox (EANx) instead of air. This double blinded, randomized trial was designed to see if the use of EANx could effectively improve cognitive performance while diving.
- METHODS:** Eight volunteers performed two no-decompression dry dives breathing air or EANx for 20 min at 0.4 MPa. Cognitive functions were assessed with a computerized test battery, including MathProc and Ptrail. Measurements were taken before the dive, upon arrival and after 15 min at depth, upon surfacing, and at 30 min postdive. After each dive subjects were asked to identify the gas they had just breathed.
- RESULTS:** Identification of the breathing gas was not possible on subjective assessment alone, while cognitive assessments showed significantly better performance while breathing EANx. Before the dives, breathing air, mean time to complete the task was 1795 ms for MathProc and 1905 ms for Ptrail. When arriving at depth MathProc took 1616 ms on air and 1523 ms on EANx, and Ptrail took 1318 ms on air and 1356 ms on EANx, followed 15 min later by significant performance inhibition while breathing air during the ascent and the postdive phase, supporting the concept of late dive/postdive impairment.
- DISCUSSION:** The results suggest that EANx could protect against decreased neuro-cognitive performance induced by inert gas narcosis. It was not possible for blinded divers to identify which gas they were breathing and differences in postdive fatigue between air and EANx diving deserve further investigation.
- KEYWORDS:** central nervous system, neuropsychology, diving safety, inert gas narcosis, Psychology Experiment Building Language.

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Research shows that success or failure in performance is influenced not only by one's abilities and limitations, but also by one's judgment. It is also widely acknowledged that one tends to act according to one's own judgment, whether or not it is the right thing to do.¹⁷ This fact may turn against us while performing underwater tasks under the influence of nitrogen narcosis.² Indeed, nitrogen narcosis, which occurs in humans at around 0.4 MPa, includes many symptoms covering a wide range of severity, starting from mild impairment of performance that can impact a diver's safety, up to hallucinations and general anesthesia.^{3,30}

Inert gas narcosis affects several neurological functions, with symptoms similar to those of alcohol poisoning, the early stages of anesthesia, or those of hypoxia.⁷ As depth increases, symptoms worsen and often become an object of mirth to divers.

Indeed, the diving community colloquially refers to this increase in severity as “Martini's law.” This “law” states that the perceived effects of inert gas narcosis are similar to those of consuming a glass of Martini every 10 to 15 m depth. However, effects of narcosis on time perception, reaction speed, and the ability to think, calculate, and respond are factors involved in many

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diving injuries, and 9% of diving fatalities are thought associated with inert gas narcosis.¹⁹

Even if most divers are acquainted with traditional depictions of narcosis, originally described by Jacques Cousteau as the “Rapture of the Deep,” it is unlikely that the recreational diver will experience “rapture.” However, there is a wide range of individual susceptibility and almost all divers will be impaired eventually. According to Bret Gilliam, a pioneering technical diver, most famous as the founder of Technical Diving International, with experience divers can learn to control these deficits to some extent, but the very real dangers should not be underestimated.⁹ Therefore, the diving community uses techniques to limit the risks associated with diving, one of them being the use of enriched air nitrox (EANx: any gas combination of oxygen and nitrogen where the oxygen fraction is greater than 21%). Because of the reduced nitrogen fraction, the main advantage of EANx diving lies in longer bottom times without additional decompression requirements compared with an air dive at the same depth. The diving community also attributes several other benefits to EANx use, such as lower gas consumption (due to the higher percentage of oxygen in the mix) and reduced severity of any barotrauma (improved circulation due to high blood oxygenation and lower nitrogen level, implying fewer or smaller nitrogen bubbles). In addition to these several unproven properties attributed to EANx, many divers report less fatigue following EANx dives compared with similar air dives.¹⁸ However, strong evidence is lacking to support this claim. To our knowledge only three studies have explored this hypothesis, with conflicting results.^{5,10,18} This double blinded, randomized controlled trial was designed to quantify nitrogen narcosis during a simulated dry chamber dive at the moderate depth of 0.4 MPa as related to the type of breathing mixture, nitrox or air, and to test the hypothesis that the reduced level of nitrogen could effectively reduce feelings of tiredness or fatigue following a dive.

METHODS

Experimental procedures were conducted in accordance with the Declaration of Helsinki and were approved by the Academic Ethical Committee of Brussels (Ethic committee B 200-2011-5). The study protocol also passed all Clinical Trial Application validation rules (EudraCT Number: 2011- 004596-37).

Subjects

All methods and potential risks were explained to eight experienced divers (minimum certification “Autonomous Divers” according to European norm EN 14,153-2 or ISO 24,801-2) with at least 50 logged dives, who gave their written, informed consent prior to the experiment. All subjects were recruited from a large sports diver population in order to obtain a group of comparable age [30–40 yr, 35.4 ± 3.6 (mean \pm SD)], body composition (BMI between 20 and 25, 23.6 ± 1.2), and comparable health status: nonsmokers with regular but not excessive physical activity (aerobic exercise one to three times a week). Prior to entry into the study, they were each assessed fit to dive. The participants

were instructed not to consume alcoholic beverages for 72 h and no caffeinated beverages for 4 h before the experiments.

Equipment

All simulated dives were performed in the hyperbaric chamber (Haux-Starmed 2800, Haux-Life-Support GmbH, Karlsbad-Ittersbach, Germany) at the Centre of Hyperbaric Oxygen Therapy, Military Hospital “Queen Astrid,” Brussels, Belgium. Each subject performed two dives on separate occasions, breathing either compressed air or EANx40 (40% oxygen–60% nitrogen) in random order, delivered via tight mask connected to the Haux-Oxymaster system that include an inspiratory and expiratory regulator. This system serves to supply and dispose of breathing gas to individual persons able to breath independently and the inhalation of exhaled gas is impossible. Both air and EANx are odorless, colorless, and tasteless, and have indistinguishable densities when breathed. Gas composition was measured using a Haux-Oxysearch (Haux-Life-Support GmbH).

Subjects and chamber attendant were blinded to the breathing gas. The chamber was pressurized with air to 0.405 MPa, equivalent to a depth of 30 msw. Bottom time was 22 min, including a 7-min compression, followed by a 12-min linear decompression ($0.033 \text{ MPa} \cdot \text{min}^{-1}$) to the surface.

Although both depth-time profile, air, and EANx fall within accepted “no-decompression limits” and oxygen toxicity limits, a 0.13-MPa/3-min safety stop was added to the dive profile.^{22,23} The chamber air temperature was maintained at $27.4 \pm 2.4^\circ\text{C}$.

Procedures

Divers were assessed for higher cognitive functions with a computerized test battery [Psychology Experiment Building Language (PEBL)] and for perceived fatigue/tiredness with a visual analog scale (VAS) immediately before the dive (baseline), upon arriving at 0.4 MPa, after 15 min at depth, when surfacing, and 30 min after surfacing. As divers were not breathing EANx either before each dive or after each dive had ended, both baseline and 30-min postdive measurements were made while breathing atmospheric air for all dives (Air and EANx). After each dive subjects were asked if, based on their experience, subjective feelings, self-evaluation of performance, or anything else, they could identify the gas they just breathed. Finally, at 30 min postdive we made a cardiac echography (Vivid-i, GE Healthcare, Chalfont St-Giles, United Kingdom) to detect the presence of vascular gas emboli (VGE).

PEBL tests were specifically chosen to track deterioration in visual-perceptual organization, visual-motor coordination and integration, and visual memory (<http://pebl.sourceforge.net/battery.html>).²¹ Four PEBL tests were used: math processing, trail making, time-wall, and perceptual vigilance. All participants underwent a short practice period before being tested in order to limit the influences of motivation, experience, and learning on the tests results.²⁰

In the math-processing task (MathProc) the participant is asked to subtract and/or add numbers of one or two digits that are presented to him on the screen and to assess whether the result is more or less than 5 in a maximum 4-s time frame; this

procedure is limited to 2 min in total. Time to complete the task within the 4-s time frame, number correct or incorrect, and timed out answers are used in the analysis.

The trail-making task (Ptrail) was used to assess brain injury, hand-eye coordination, and general intelligence. The test is divided into two parts. In the first part, the circles are numbered (1, 2, 3, etc.) and the test subject has to connect them in numerical order (1, 2, 3, etc.). In the second part, the circles have both numbers and letters and the subject has to click on them in alternating order (1-A, 2-B, 3-C, etc.). The trial continues until the test person has connected all the circles in the correct order. The number of clicks to finish the test and erroneous clicks were counted for analysis.

Time-wall (Twall) is a basic time/movement estimation task in which a moving object disappears behind a wall and the participant must judge when it would have reached a gap. The primary dependent measure is inaccuracy, defined as the absolute value of the participant's response time minus the correct time divided by the correct time for that trial. Since the majority of responses on tests of this type are too early,²⁷ the percentage of trials on which response time was greater than the correct time was determined. These two values, which map roughly onto precision and bias, were used for the analysis.

The perceptual vigilance task (PVT) test is commonly used to measure simple response time. Using a computer screen and a keyboard, the participant has to press the spacebar as soon as possible when a red circle stimulus appears randomly, at delays between 2 and 12 s, 16 times. The reaction time is captured for analysis.

Fatigue was assessed using a 100-mm VAS. In order to test the attention and comprehension of the diver, the same scale was presented twice but in opposite directions: one asked to evaluate the 'energy level' (from sleepy/0 to energetic/10), the second asked to evaluate the 'tiredness level' (from energetic/0 to sleepy/10). Only if the scores on both scales were coherent was the result considered valid.

Statistical Analysis

Because of the design of the PEBL (timed experiment), depending on the speed of the participant, each performed a different

number of calculations (MathProc), simple response time (PVT), or time/movement trial (Twall). Before performing statistical analysis, we calculated the mean for each test and participant in order to obtain a unique set of eight measurements for each condition. For MathProc and Ptrail, taking the pre-dive values as 100%, percentage changes were calculated for each parameter, allowing an appreciation of the magnitude of change between each measurement rather than the absolute values. Since all data passed the Kolmogorov-Smirnov and Shapiro-Wilk tests, allowing us to assume a Gaussian distribution, they were analyzed with repeated measures of ANOVA. Differences between air and EANx dives in post-dive estimates of fatigue (VAS) were tested with a two-tailed *t*-test.

To assess the accuracy of the divers' ability to identify which gas they had been breathing, a ternary logistic model was constructed using a cumulative logit function appropriate for ordinal, polychotomous dependent variables. All PEBL statistical tests were performed using a standard computer statistical package, GraphPad Prism version 5.00 for Windows (GraphPad Software, San Diego, CA). The logistic regression was performed with SAS version 9.3 (Cary, NC). A threshold of $P < 0.05$ was considered statistically significant in all cases. All data are presented as mean \pm SD.

RESULTS

The perceptual vigilance task did not show a significant difference between gas and time ($P = 0.06$, one-way ANOVA with Bonferroni multiple comparison test, $df = 9$). The evolution of both MathProc and Ptrail during and after the dive is illustrated in Fig. 1. Since error rate was stable throughout the experiment in both MathProc (Air: $8.4 \pm 1.0\%$ vs. EANx: $8.9 \pm 1.4\%$; $P = 0.99$, one-way ANOVA with Bonferroni multiple comparison test, $df = 9$) and Ptrail (Air: $1.0 \pm 0.0\%$ vs. EANx: $1.0 \pm 0.0\%$; $P = 0.38$, one-way ANOVA with Bonferroni multiple comparison test, $df = 9$) independently of the nature of the gas breathed, only mean time to completion is presented.

Before the dives, the mean time to complete the task was 1795 ± 750 ms (MathProc) and 1905 ± 657 ms (Ptrail). Evolution is characterized by significant

decrease of time to completion when arriving at depth in both tests; MathProc (Air: 1616 ± 612 ms; EANx: 1523 ± 694 ms) and Ptrail (Air: 1318 ± 314 ms; EANx: 1356 ± 852 ms), followed 15 min later by an increase in air diving (and slightly in EANx diving for MathProc times). This increase in time to complete corresponds to gradual inhibition while breathing air ($P < 0.0001$, one-way ANOVA with Bonferroni multiple comparison test, $df = 9$, compared with EANx) in both Mathproc (surfacing: 1858 ± 581 ms; post-dive: 1865 ± 685 ms, Fig. 1A) and Ptrail (surfacing:

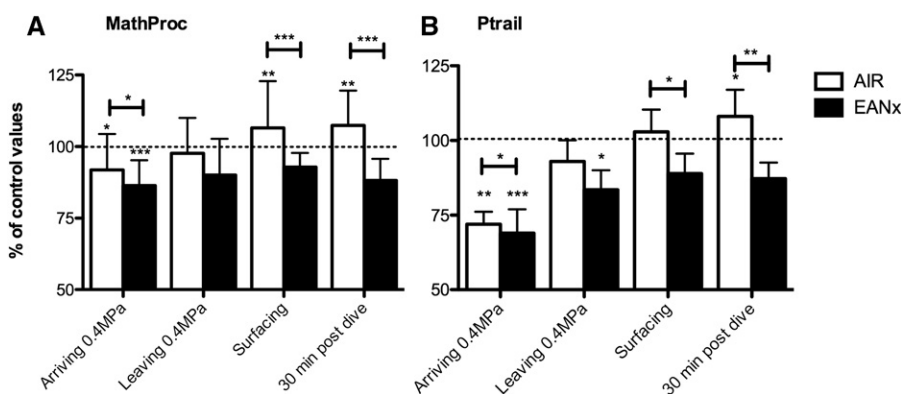


Fig. 1. Variation of time to complete (%) in A) Mathproc and B) Ptrail during and after a 22-min dry chamber dive to 0.4 MPa. Pre-dive value is taken as 100%. Each subject is compared to his own pre-dive value. Error bars indicate standard deviation, *** $P < 0.001$; ** $P < 0.01$; * $P < 0.05$; $N = 8$.

1989 ± 665 ms; postdive: 1997 ± 397 ms, Fig. 1B). Compared with pre-dive values, in EANx breathing mean times were significantly lower when leaving 0.4 MPa (MathProc 1527 ± 659 ms, Ptrail 1458 ± 386 ms), surfacing (MathProc 1575 ± 697 ms, Ptrail 1619 ± 746 ms), and at 30 min postdive (MathProc 1495 ± 639 ms, Ptrail 1549 ± 411 ms).

On average, time wall estimation was 273 ± 37 ms early and the average inaccuracy was constant at 7.3 ± 0.7% independent of the gas breathed ($P = 0.06$, one-way ANOVA with Bonferroni multiple comparison test, $df = 9$; Fig. 2). However, air dives were characterized by later responses, especially during the ascent and in the postdive phase ($P < 0.0001$, one-way ANOVA with Bonferroni multiple comparison test, $df = 9$), supporting the idea of late dive/postdive impairment while breathing air (leaving: 44 ± 25%; surfacing: 51 ± 28%; postdive: 43 ± 23%). Though the effect of breathing air upon late responses was significantly greater than when breathing nitrox at both the time of leaving 0.4 MPa and upon surfacing, both air and nitrox were significantly different at 30 min postdive compared with baseline values, though not significantly different from each other.

Fig. 3 shows the results of the VAS evaluation. It can be seen there is a trend toward increased perceived fatigue, which is significantly higher between the air dives compared with EANx dives immediately after surfacing ($P < 0.001$, two-tailed t -test, $df = 19$). However, both postdive measurements were significantly different from pre-dive values ($P < 0.01$ and < 0.05 , one-way ANOVA with Bonferroni multiple comparison test, $df = 9$, for air and EANx, respectively).

Although divers were blinded at all times to the gas breathed, we asked each participant if they could, based on their experience, subjective feelings, self-evaluation of performance, or anything else, identify the gas they had just breathed. For the purpose of analysis, when a diver hesitated or reversed their initial call after the second dive, we considered these answers to lie somewhere between Right and Wrong, and so they were collapsed into a middle level outcome (Fig. 4). Oxygen content was not significantly associated with the ability to identify which gas

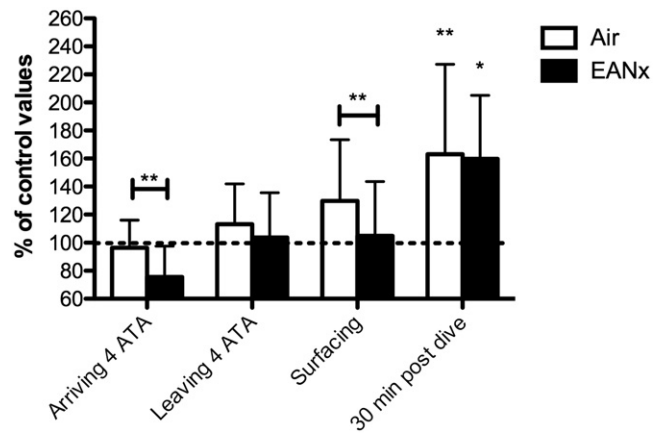


Fig. 3. Percentage variation of VAS during and after a 22-min dry chamber dive to 0.4 MPa. Pre-dive value is taken as 100%. Each subject is compared to his own pre-dive value. Error bars indicate standard deviation, ** $P < 0.01$; * $P < 0.05$, $N = 8$.

was enriched ($P = 0.74$, Wald Chi-square, $df = 1$). Neither gas was identifiable significantly more (or less) often than the other. Finally, we were not able to detect any circulating bubbles during the postdive echocardiography.

DISCUSSION

One solution adopted by divers to limit either narcosis or postdive fatigue is to use a reduced fraction of nitrogen in the breathing mixture, either EANx or, for deep diving, Trimix (a breathing mixture containing oxygen, nitrogen, and helium). However, studies evaluating the cognitive effect of air diving vs. EANx diving have produced conflicting results. In the first study with 3500 EANx 32% dives conducted over a 3-mo period, the authors noted that many of the divers reported being less fatigued after surfacing compared with divers breathing air.⁵ However, in this study a major bias was likely, since EANx divers had shorter total immersion and decompression times than the air dives with which they were compared, casting doubts over the conclusions. In the second study,¹⁰ a simulated dive in a hyperbaric chamber was performed, controlled for depth, bottom time, decompression

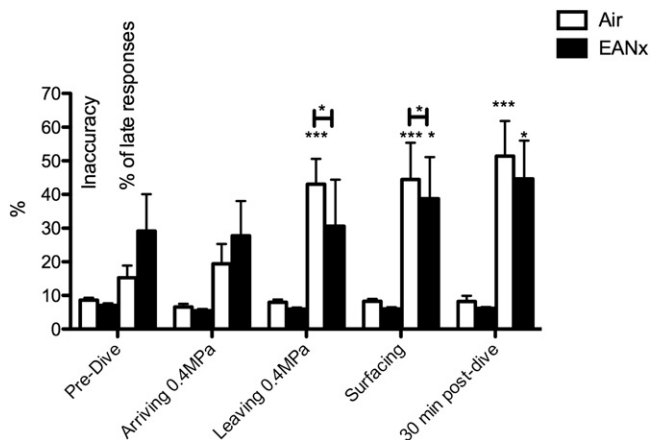


Fig. 2. Time-wall inaccuracy and percentage of late response during and after a 22-min dry chamber dive to 0.4 MPa. Error bars indicate standard deviation, *** $P < 0.001$; * $P < 0.05$; $N = 8$.

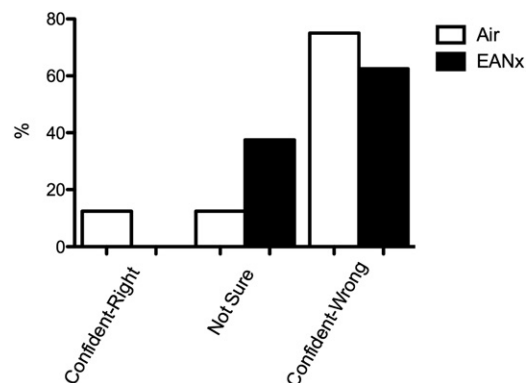


Fig. 4. Diver identification of the breathed gas according to their self-assessment of performance (subjective evaluation).

rate, temperature, and physical exertion. In this experiment, breathing air produced no measurable difference in fatigue, attention levels, or ability to concentrate compared with EANx 36%. However, even with the “air dive,” fatigue did not increase at all. This may be related to the “shallow exposure” (0.28 MPa). Also, the authors, as had others,^{13,26} agreed that simulated dives probably differed in many respects to actual underwater diving; they thus expressed a need for reliable in-the-field measurement.

Compared with underwater diving, simulated dives lack the effects of immersion upon the peripheral vasculature, heat loss, movement resistance, and other physiological effects. Finally, in a large group of divers, using the VAS, EANx dives did not seem to provoke any postdive increase in perceived fatigue. On the other hand, after air dives, perceived postdive fatigue increased significantly.¹⁸ In the same study, objective evaluation of brain performance with measurements of critical flicker fusion frequency also showed impairment with air breathing, but slight improvement with EANx.

This discrepancy may probably be explained by the use of evaluation methods based on the diver's subjectivity. Given our results (Fig. 4), it seems that the subjective assessment does not meet the criteria of reliability. Indeed, when blinded as to the nature of the breathing gas, the divers, based on self-assessment of performance, were unable to identify the gas used, either because they could not reliably distinguish between the two gasses or they could tell a difference, but without being able to identify them correctly. Yet the VAS, a widely used and validated measure of subjective sensations such as pain and fatigue, appears an ideal tool to quantify and compare self-reported fatigue levels,^{12,32} and is consistent with cerebral performance evaluation made by the PEEL.

Consequently, there exists a need to objectively test neuro-cognitive performance during immersed diving. However, some methodological points of our study need to be raised. Although the behavioral approach confirms the progressive inhibition of cognitive performance in parallel with exposure to pressure while breathing air, these methods have been criticized because of the influence of motivation, experience, and learning that can improve performance in tested tasks.^{2,4} However, outside the Ptrail, whose average time to complete the trace steadily decreases with practice, the other tests were selected for their resistance to learning and practice. Indeed, data published on the MathProc show that the effect tends to stabilize after the first round of tests (eight tests) and a limited practice period has, therefore, little effect on the results.¹ For the PVT and Twall, it has been shown that motivation can counteract the negative effects of sleep deprivation up to 36 h.¹⁴ However, this effect was controlled for by the design of our study. Indeed, each candidate being his own control and with the test phase not exceeding 45 min, the effect of motivation was smoothed with respect to changes in time, which was our most important criterion for analysis. Therefore, we began the experimental session after only two practice tests.

Our results indicate that the second practice test and baseline test showed no significant difference in completion

time ($P = 0.75$) across all subjects. There was also no difference in time of completion between baseline tests before each experimental condition (air and EANx), which were recorded on separate occasions and in random order. This suggests that any learning process was either completed before, or did not take place during, the experiment. In any case, it seems that learning did not influence our results.

Another point to be raised is that there is a risk that accuracy may be sacrificed in an attempt to maintain the speed of response.²⁸ This was not the case in the present study as participants were instructed to be as quick as possible but with a minimal error rate. Since error rate on our study remained constant throughout measurement, independently of the gas breathed, our conclusions may be based on completion time only. To explain the difference in perceived fatigue between Air and EANx dives, three hypotheses are to be considered: the potential effect of bubbles, nitrogen, and oxygen.

The first hypothesis to be considered is the potential effect of circulating bubbles. Even if postdive fatigue is multifactorial, it is nonetheless listed as an important symptom in the list of stress events or decompression sickness.^{15,25} Reduced fatigue after diving by using EANx suggests a pathological origin of this fatigue, attributed to the presence of asymptomatic nitrogen bubbles in the body after a dive.¹⁶ Indeed, decompression profiles that have a high K value ($K = \text{speed of decompression/inspired oxygen partial pressure}$) generate more decompression stress.²⁹ However, we did not detect any bubbles with ultrasound.

Before exploring the effect of the gases, it should be remembered that these effects are directly related to the amount of dissolved gas in the tissues, which depends on the partial pressure of each gas as defined by Dalton's law and exposure as defined by Henry's Law. Although it remains largely theoretical, there is a way to model the evolution of these partial pressures.^{24,33} Upon arrival at depth, inspired gasses would dissolve into the bloodstream via pulmonary circulation and be carried to the well-perfused brain, where they would diffuse into tissue according to Fick's First Law of Diffusion.

Oxygen, however, would be additionally transported via hemoglobin and, therefore, would reach equilibrium sooner than nitrogen. This serves to explain why cognitive performance improved upon arrival at depth, followed by a return to baseline in air breathing. This corresponds to the peak of performance across all measures, suggesting either an effect of increased PO_2 or relatively lower PN_2 (compared with air dives). Indeed, a previous study using critical flicker fusion measurements, before and after oxygen breathing in nondivers, supported the effect of oxygen on cerebral arousal.¹¹ Critical flicker fusion frequency increased by almost 25% compared with baseline measurements. This same effect could be responsible for the increased critical flicker fusion frequency observed in the beginning of the dive. While at 33 m depth, divers breathing air breathe a PO_2 of 0.8 ATA, which approaches breathing pure oxygen at the surface. It could also explain the effect of postdive oxygen breathing, as the critical flicker fusion frequency at 30 min postdive increased 24%.

The two most remarkable observations are undoubtedly that, firstly, cognitive performance inhibition identified by both the PEBL and the VAS was preceded by brain activation regardless of breathing air or nitrox and, secondly, that any performance inhibition observed at depth while breathing air may persist until at least 30 min after surfacing. This would be a logical observation regarding the proteic theory of narcosis, where nitrogen acts directly as a drug on dopaminergic neurons through GABA receptors.³¹ Indeed, anesthesia and inert gas narcosis likely share the same mechanisms. However, based on the study of Colloc'h *et al.* using X-ray crystallography to examine the behavior of xenon and nitrous oxide,⁶ we can assume a stepwise mechanism in which the graded dose-response curve would depend on the size of the effect site and the order of occupation. Gaseous anesthetics will first bind to brain intracellular proteins that have large hydrophobic cavities and are, therefore, easy targets. These bonds inhibit the activity of these proteins in a manner sufficient to induce moderate neuronal dysfunction and lead to the early stages of anesthesia (hypnosis and amnesia). If the gas concentration increases, smaller effect sites are then affected, thereby increasing the dysfunction of N-methyl-D-aspartate (NMDA) receptors and resulting in surgical anesthesia. Similar mechanisms, which assume a causal relationship between the behavioral effects of anesthesia and the gradual occupation of the binding sites of membrane proteins, can occur for other types of inhaled anesthetics or narcotic gas and/or receptors such as gamma-aminobutyric acid (GABA) receptors, regarded as the molecular target of nitrogen and oxygen.¹¹ The net effect on brain arousal and related critical flicker fusion frequency measurements (activation followed by a sustained impairment even after surfacing) would then result from a balance between the direct “drug” effect of the different gases, nitrogen and oxygen, on the GABA receptors and the pharmacokinetics of these interactions.

This last point is crucial for optimal diver safety. Indeed, based on the lipid theory,³⁴ diver training programs advise that in the event of nitrogen narcosis, divers should ascend a few meters in order for the narcotic effects to dissipate rapidly.⁸ However, we show here that, even if subjective sensations of narcosis may decrease quickly, subjective sensation cannot be trusted, as cerebral impairment persists for at least 30 min after surfacing. This would be an important consideration in situations where precise and accurate judgment or fast actions are essential, such as in hazardous situations in recreational or professional (industrial, military) diving.^{2,18}

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