Physiological and Cognitive Effects of Acute Normobaric Hypoxia and Modulations from Oxygen Breathing

Carine Malle; Cyprien Bourrilhon; Peggy Quinette; Mickaël Laisney; Francis Eustache; Christophe Piérard

INTRODUCTION: The emergence of normobaric devices for hypoxia awareness training makes crucial the study of physiological and cognitive effects induced by acute normobaric hypoxia (NH) exposure. Our study aimed to 1) investigate the effects of acute NH exposure on physiological variables and working memory; and 2) investigate the physiological and cognitive effects of oxygen breathing before and after acute NH exposure.

METHODS: There were 86 healthy men who were randomized into 4 groups: the Normoxia-Air group ($N = 23$), whose subjects were breathing air; the Hypoxia-Air group ($N = 22$), where NH exposure was preceded and followed by air breathing; the Normoxia-O_2 group ($N = 21$), whose protocol was similar to the Normoxia-Air group, except with the addition of 100% O_2 breathing periods; and the Hypoxia-O_2 group ($N = 20$), whose participants were exposed to 100% O_2 before and after NH exposure. Working memory was assessed with the Paced Auditory Serial Addition Test. Peripheral oxygen saturation (S_pO_2), heart rate (HR), and electroencephalogram (EEG) were recorded.

RESULTS: Acute NH exposure induced a classical physiological response (i.e., decreased S_pO_2 and increased HR), but not identical to the well-described physiological response to acute hypobaric hypoxia. Acute NH also caused a strong impairment in working memory. Oxygen breathing following NH exposure induced a slowing in the EEG associated with a worsening of working memory performance.

DISCUSSION: Acute NH exposure revealed a good surrogate for the classical hypobaric chamber for refresher hypoxia awareness training. Because the association between hypoxia and hyperoxia seems deleterious for the brain, we suggest that NH exposure should be surrounded by air breathing.

KEYWORDS: altitude, aeromedical training, working memory, Paced Auditory Serial Addition Test, electroencephalography.

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Hypoxia still remains one of the most serious single hazards during flight at altitude.[10] Because in-flight hypoxic incidents can result in death, hypoxia awareness training is a mandatory part of aeromedical training for military aircrews around the world. Aircrews who have been trained about hypoxia respond better to in-flight hypoxic incidents,[7] reinforcing the need for hypoxia awareness training. Military aircrew are trained to recognize the signs and symptoms of acute hypoxia using a variety of methods to simulate high altitude exposure. Since the late 1930s, hypobaric hypoxia (HH) has been traditionally used for military aviation hypoxia training around the world. Over the last two decades, new generation normobaric hypoxia (NH) training devices have been introduced as alternatives to the traditional hypobaric chamber. Hence, the U.S. Navy routinely uses a reduced oxygen breathing device (ROBD) for refresher training of fast tactical jet aircrew. This change is often motivated by practical reasons: NH devices are simpler to use, less cumbersome, and require less maintenance. Medical reasons are also used, since there is no

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decompression sickness (DCS) risk with NH. Plus, it has been recently suggested that high-altitude (but nonhypoxic) hypobaria could induce cerebral white matter lesions.[15]

The human physiological response to acute NH in terrestrial conditions [simulated altitudes below 5000 m (16,404 ft)] has been extensively studied. There is a growing body of evidence suggesting that physiological response to acute hypoxia may differ between NH and HH conditions, although these results are still very controversial.[17] In particular, Savourey et al.[22] showed that HH leads to a distinct ventilatory response. As far as we know, the human systemic physiological response to acute NH exposure, equivalent to fast jet flight levels [below 7200 m (25,000 ft)], has not been described earlier. This preliminary stage seems necessary before routinely exposing aircrew to acute NH in the context of aeromedical training. Moreover, because hypoxia awareness training relies mainly on familiarization with hypoxia symptoms, it is crucial that NH conditions elicit the same symptoms that a "real-life" HH environment would. In a study comparing the effects of NH and HH exposure, Self et al.[24] found similar patterns in symptom frequencies, including subjective cognitive impairment, in both conditions. This finding suggests that NH training may be a relevant surrogate for HH training. To our knowledge, there is no published study comparing objective cognitive effects induced by HH and NH. In a previous study,[14] we showed that an acute exposure to HH, at a simulated altitude of 10,000 m (32,808 ft), induces a strong, transient impairment of working memory. To our knowledge, there is only one published work concerning the effects of acute NH on working memory.[8] The authors showed that acute NH exposure [equivalent to 4200 m (13,780 ft)] was responsible for an impaired performance in both a dichotic listening task and a scanning task that was attributed to an attentional deficit. Therefore, the question of the impact of an exposure to a more aeronautical altitude [around 10,000 m (32,808 ft)] on working memory is still not completely answered.

Another question concerns the need to expose the subjects to 100% oxygen (O_2) before and after acute NH exposure. In acute HH, preoxygenation, also referred to as denitrogenation, is aimed at reducing the risk of DCS by removing nitrogen (N_2) from the body. Thus, the NATO Standardization Agreement (STANAG) 7056 AMD[18] states that a 45-min preoxygenation period must be performed before an exposure to a simulated altitude (acute HH conditions) between 25,000 and 30,000 ft (7620 and 9144 m). In NH, preoxygenation could be used to increase the delay before desaturation occurs. According to Tanoubi et al.,[27] a preoxygenation period lasting 3 min is needed to reach an expired fraction of oxygen (F_EO_2) greater than 90%. One can also wonder if oxygen would promote or worsen the recovery from NH (in comparison to air) since there is a growing body of evidence suggesting that postresuscitation hyperoxia (e.g., in the medical management of cardiac arrest) could result in brain injuries.[2] This study aimed to 1) investigate the effects of acute NH exposure on physiological variables and working memory; and 2) investigate the physiological and cognitive effects of oxygen breathing before and after acute NH exposure.

METHODS

Subjects

The study was performed at the Institut de Recherche Biomédicale des Armées in Brétigny sur Orge, France. There were 86 healthy young (29.4 ± 0.9 yr in average) men who participated in this study. Participants were randomized into the four following groups: the Normoxia-Air group ($N = 23$), where subjects were breathing air during the whole experiment; the Hypoxia-Air group ($N = 22$), where hypoxia exposure was preceded and followed by air breathing; the Normoxia-O_2 group ($N = 21$), with the same protocol as the Normoxia-Air group, except with the addition of two 3-min 100% oxygen breathing periods (it should be noted that this group was only used to check any influence of breathing periods by themselves and does not appear in the following analyses); and the Hypoxia-O_2 group ($N = 20$), where participants were exposed to 100% oxygen before and after hypoxia exposure. In accordance with Tanoubi et al.,[27] 3-min breathing periods were used. Each group's experimental schedule is presented in **Fig. 1**. The study protocol followed the tenets of the Declaration of Helsinki and was approved in advance by an external ethics committee (Comité de protection des personnes Ile-de-France VI, ID RCB: 2010-A01265-34). Each subject was briefed in detail and provided written informed consent before participating.

Equipment

The elevation of the laboratory where the NH exposure was performed is approximately 78 m (260 ft; 1003.9 hPa). For inducing acute NH, we used three high-pressure bottles that varied in oxygen, with the balance being nitrogen: a 100% O_2 bottle, a 21% O_2 bottle (ambient air), and a 6% O_2 bottle (hypoxic mixture). These bottles were linked to a command that allowed for the manual selection of any one of them. A pressure regulator placed downstream reduced the pressure to ambient level. Subjects breathed through an aeronautical mask (Ulmer Aeronautique, Bobigny, France). Systemic physiological variables, comprising arterial blood oxygen saturation (S_pO_2), heart rate (HR), and electroencephalogram were continuously displayed and recorded online on a personal computer using a data acquisition system (Model ML870; PowerLab, ADInstruments, Sydney, Australia) with LabChart (Version 6; ADInstruments). S_pO_2 and HR were measured by pulse oximetry (Radical-7, Masimo Corporation, Irvine, CA). The pulse oximetry sensor was placed on the subject's forefinger. The sampling frequency was 256 Hz. Mean HR and mean S_pO_2 were calculated during each phase of hypoxia exposure (these phases will be described later). HR variation and S_pO_2 variation were also calculated during each phase (difference between the end value and the start value, so that a positive number corresponds to an increase and a negative number corresponds to a decrease in the variable during the phase). Because electroencephalography (EEG) has a very good temporal resolution, it is a suitable tool to study the temporal aspects of neural activity correlated with simultaneous cognitive performance. EEG was recorded from three scalp locations from the international 10–20 system

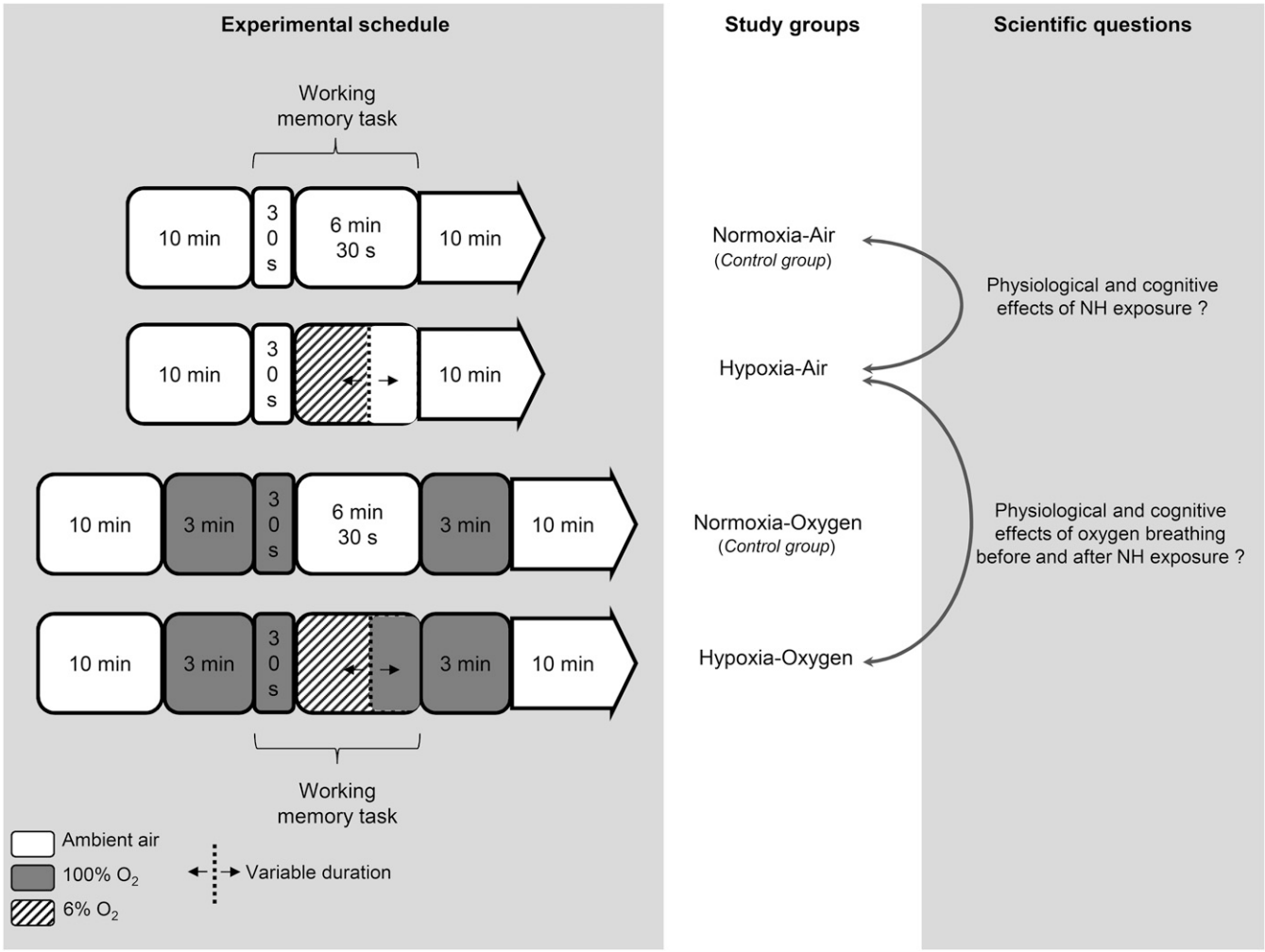

Fig. 1. Each group's experimental schedule was composed of several sequences (rectangles with rounded corners) of ambient air (white), 100% O_2 (gray), or 6% O_2 (line patterned) breathing whose duration is indicated inside the rectangle. The dotted line surrounded by arrows illustrates the variable duration of the 6% O_2 mixture breathing phase.

(channels F4, C4, and O2), with all electrodes referenced to the chin. EEG recordings were sampled at 1000 Hz and band-pass filtered (low-pass filter cut-off frequency: 40 Hz, high-pass filter cutoff frequency: 0.5 Hz). The Infomax Independent Component Analysis (ICA) technique was applied to remove artifacts from the data such as eye movements, eye blinks, cardiac signal, and muscle noise.[13] The analysis and extraction of the artifacts was performed off-line on a PC by means of the EEGLAB software coded in MATLAB.[5] Additionally, EEG measurements were divided into 4-s segments (interstimulus interval of the working memory task). The power spectrum of each EEG segment was estimated from the modified periodogram as computed from the Fast Fourier Transform (FFT). The absolute and relative powers for the classic frequency bands of EEG were estimated: delta (2–4 Hz), theta (4–8 Hz), alpha (8–12 Hz), beta-1 (12–18 Hz), and beta-2 (18–30 Hz). SEF95, i.e., the frequency below which 95% of total EEG power was contained, was also calculated. This index has been shown to be useful in assessing the relative change in lower frequency activity (typically delta and theta) and/or in higher frequency activity (typically alpha and beta).[16] The first 30 s of the working memory task were used for baseline measurements. In the interest of clarity, we only present here the data recorded from the F4 electrode.

Design

The study design is described in Fig. 1. In both hypoxic groups, hypoxic exposure stop criteria were: subject's demand, major behavior disturbance (e.g., incoherent speech), cardiac rhythm disorder (evidenced on the electrocardiogram), appearance of cerebral delta waves (evidenced on the electroencephalogram), and arterial blood oxygen saturation lower than 60%. The time between air/O_2 removal and air/O_2 restoration was defined as the Time of Useful Consciousness (TUC). The comparison of the Normoxia-Air group and the Hypoxia-Air group allowed the investigation of the cognitive impact of NH. The analysis method consisted of drawing the mean blood oxygen saturation's curve of the Hypoxia-Air group and then dividing this curve into several phases (which are described in the Results paragraph). Then we calculated mean systemic physiological measurements and mean working memory performance

during each phase in the Hypoxia-Air and Normoxia-Air groups. The comparison of the Hypoxia-Air group and the Hypoxia-O_2 group allowed the study of the physiological and cognitive effects of oxygen breathing before and after NH exposure in comparison to air breathing. For this analysis, we focused on NH exposure (from air/O_2 removal to air/O_2 restoration) and on NH recovery (from air/O_2 restoration to the normalization of blood oxygen saturation). NH exposure and recovery were both divided into 16-s intervals and mean physiological measurements and mean working memory performance during these intervals were calculated.

Procedure

The working memory task consisted of a modified version of the Paced Auditory Serial Addition Task (PASAT[9]). Our version of the PASAT comprised more items (105 items instead of 61 in the original version) and the interstimulus interval was lengthened (4 s instead of 2 or 3 s). In our previous study of HH,[14] we used the original 61-item version, but the interstimulus interval was already 4 s. This longer version (7 min) enabled us to cover the whole acute hypoxia exposure and recovery period. The PASAT is one of the tests most frequently used by neuropsychologists to assess attentional processing and working memory. In this study, the PASAT was chosen for two major reasons: 1) it is a serial test that enables correlations with EEG activity over the time; and 2) it is highly sensitive to hypoxia (as showed in our previous study of HH[14]). Administration of the PASAT involves presenting a series of single-digit numbers where the two most recent digits must be summed. For example, if the digits '3', '6', and '2' were presented, the participant would respond with the correct sums, which are '9' and then '8'. The participant must respond prior to the presentation of the next digit for a response to be scored as correct. Errors were categorized into three types: omissions (no response), suppression failures (i.e., adding the digit to the sum of the last addition rather than to the last heard digit), and miscalculations (other errors). The mean delay between errors corresponds to the mean time between two errors or two strings of errors if there are consecutive errors. Moreover, trait and state anxiety were assessed 15 min before hypoxia induction with the State-Trait Anxiety Inventory (STAI[25]).

Statistical Analysis

Data analysis was performed using SigmaPlot (SigmaPlot 12.0, Systat Software Inc., San Jose, CA). An unpaired two-sample Student's *t*-test was used to compare the mean TUC durations in the Normoxia-Air and Hypoxia-Air groups. A one-way repeated measures analysis of variance (ANOVA) was used to investigate significant changes in physiological and cognitive measurements over time in each group. A two-way ANOVA was used to examine statistically significant differences in S_pO_2, HR, and working memory measurements between groups over the experimental phases. The two independent variables were the group and the experimental phase. A post hoc analysis was performed in order to reveal the phases with between-groups differences. Last, a two-way ANOVA was used in order to investigate statistically significant differences in EEG and working memory measurements between experimental conditions over time during NH exposure and recovery. The two independent variables were the group and the period of time. A post hoc analysis was performed in order to reveal the periods of time with between-groups differences. A two-tailed *P*-value of smaller than 0.05 was considered significant. Data are presented as mean ± SEM.

RESULTS

All groups were matched in age [29.4 ± 0.9 yr on average; $F(3,82) = 0.77$; $P = 0.52$], school level [15.1 ± 2.4 yr on average; $F(3,82) = 0.97$; $P = 0.58$], Epworth Sleepiness Scale (ESS[12]) score [5.2 ± 2.0 on average; $F(3,82) = 3.48$; $P = 0.34$], trait anxiety score [34.6 ± 1.9 on average; $F(3,82) = 1.01$; $P = 0.26$], and state anxiety score [31.1 ± 1.7 on average; $F(3,82) = 4.84$; $P = 0.29$].

Systemic Physiological Response and Cognitive Response to NH Exposure

The mean S_pO_2 and HR curves of the Hypoxia-Air and of the Normoxia-Air groups are shown in **Fig. 2**. We used the Hypoxia-Air group's S_pO_2 curve to define six phases: 1) the control phase, i.e., the first 30 s of the PASAT, performed in normoxia/hyperoxia; 2) the desaturation-delay phase, which was the time between air/O_2 removal and the beginning of the decrease in S_pO_2 (2% S_pO_2 decrease); 3) the desaturation phase, i.e., the period of time between the beginning of the decrease in S_pO_2 and the end of hypoxia exposure; 4) the recovery-delay phase, which was the interval between air/O_2 restoration and the increase in S_pO_2; 5) the recovery phase, i.e., the period of time between the beginning of the increase in S_pO_2 and the normalization of S_pO_2 (98%); and 6) the stabilization phase, which is the period of time between S_pO_2 normalization and the end of PASAT administration. In order to perform between-group comparisons, the mean duration of each phase in the Hypoxia-Air group was used to define equivalent phases in the Normoxia-Air group. During the control phase, mean S_pO_2 [$F(1,43) = 0.594$; $P = 0.445$] and mean HR [$F(1,43) = 0.911$; $P = 0.345$] were equivalent in both groups, as was the increase in HR (22 ± 4 bpm in the Normoxia-Air group and 21 ± 3 bpm in the Hypoxia-Air group [$F(1,43) = 0.026$; $P = 0.873$].

The desaturation-delay phase lasted 36 ± 2 s. During this phase, mean S_pO_2 [$F(1,43) = 0.056$; $P = 0.829$] and mean HR [$F(1,43) = 0.047$; $P = 0.829$] remained equivalent in both groups. The desaturation phase lasted 127 ± 5 s. Therefore, the TUC (desaturation delay phase + desaturation phase) lasted 163 ± 3 s. Mean S_pO_2 at stop was 61.5 ± 1.0%, which corresponds to a mean decrease of −38.4 ± 1.0%. Mean HR was higher [$F(1,43) = 15.661$; $P < 0.001$] and increased (+18 ± 2 bpm) in the hypoxic group while decreasing in the normoxic group [−7.6 ± 2.0; $F(1,43) = 25.072$; $P < 0.001$].

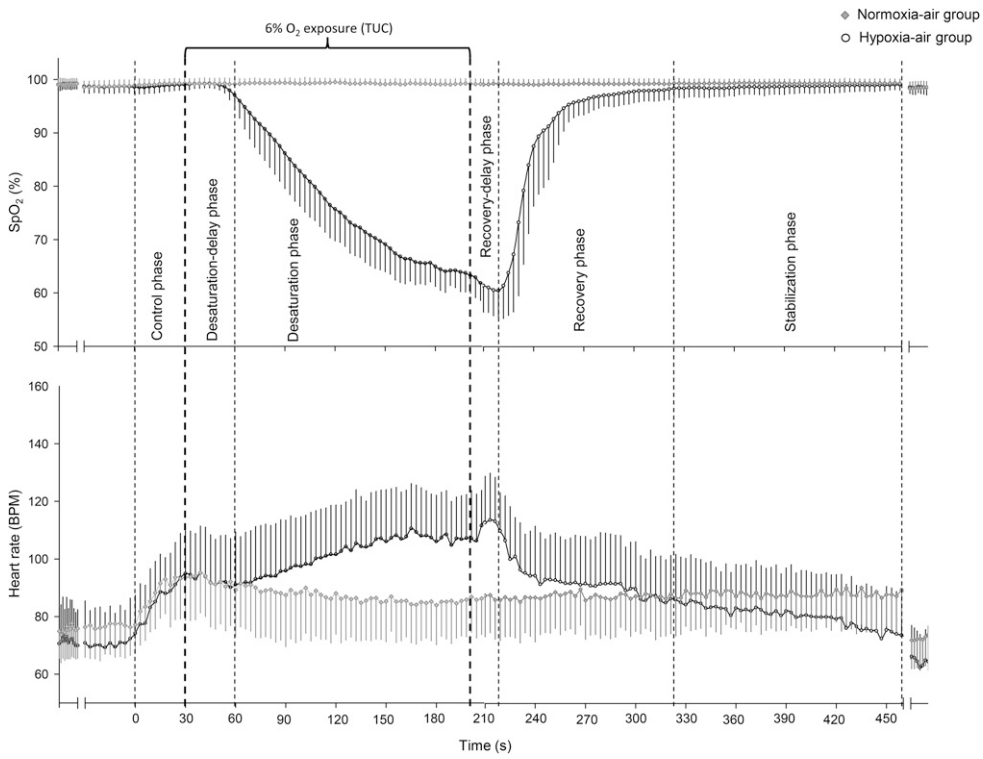

Fig. 2. Mean S_pO_2 (%; top graph) and mean heart rate (bpm; bottom graph) over time (s) in the Normoxia-Air (gray diamonds connected with gray line) and Hypoxia-Air groups (white circles connected with black line). Error bars are SEM.

The recovery-delay phase lasted 20 ± 2 s. During this phase, S_pO_2 reached a minimum value of 58.5 ± 1.1% in the hypoxic group, which represent a mean decrease of 1.6 ± 0.6% [significantly different from the normoxic group; $F(1,43) = 12.142$; $P = 0.001$], and mean HR was still higher in the hypoxic group [$F(1,43) = 20.710$; $P < 0.001$]. The recovery phase lasted 100 ± 13 s. During this phase, mean S_pO_2 remained lower in the hypoxic group [$F(1,43) = 12.946$; $P < 0.001$], whereas mean HR became equivalent in both groups [$F(1,43) = 0.843$; $P = 0.364$]. During this phase, the mean increase in S_pO_2 was 38.2 ± 1.1% in the hypoxic group [significantly different from the normoxic group; $F(1,43) = 38.198$; $P < 0.001$]. HR decreased in both groups, but the drop was significantly higher in the hypoxic group [$F(1,43) = 15.116$; $P < 0.001$]. During the stabilization phase (126 ± 22 s), similarly to the control phase, there was no between-group difference in mean S_pO_2 [$F(1,43) = 0.370$; $P = 0.545$] and in mean HR [$F(1,43) = 1.307$; $P = 0.259$].

The PASAT results in the Hypoxia-Air and Normoxia-Air groups were also analyzed in a phase-by-phase way. The main results are summarized in **Table I**. No between-group differences were noticed either during the control phase or during the desaturation-delay phase. During the desaturation phase, the percentage of correct responses became lower in the hypoxic group (70.3 ± 3.9% vs. 88.2 ± 2.1%). During this phase, the hypoxic group made both more omissions and miscalculations. However, the percentage of suppression failures was similar between groups. The analysis of errors did not show any significant difference between the error patterns. Interestingly, we showed that errors were almost twice more frequent in the hypoxic group (12 ± 1 s between errors vs. 23 ± 3 s). Because of the low duration of the recovery-delay phase (20 ± 2 s), we merged the recovery-delay phase and the recovery phase. During these phases, the percentage of correct responses remained lower in the hypoxic group (80.0 ± 2.8% vs. 88.2 ± 2.2%). In addition, the hypoxic group made more omissions. However, the percentages of miscalculations and suppression failures were similar between groups. Like during the desaturation phase, no significant difference was found between the error patterns. During this phase, the delay between errors became equivalent in both groups. During the stabilization phase, similarly to the control phase, no between-group differences were noticed.

Physiological and Cognitive Effects of Oxygen Breathing Before and After NH Exposure

The following results involve the comparison between the Hypoxia-Air group and the Hypoxia-O_2 group. We performed a phase-by-phase analysis of S_pO_2 and HR measurement results in the Hypoxia-Air and Hypoxia-O_2 groups. The main results are summarized in **Table II**. Except for the mean S_pO_2 value, no between-group differences were noticed in any of the phases. The duration of the experimental phases surrounding the desaturation phase were different between groups. Indeed, the desaturation-delay phase was significantly longer in the Hypoxia-O_2 group (89.6 ± 5.0 s vs. 36 ± 2 s). As a result, the TUC was also significantly longer in the Hypoxia-O_2 group (230 ± 5 s vs. 163 ± 3 s). Similarly, the recovery-delay phase was significantly shorter in the Hypoxia-O_2 group (13 ± 1 s vs. 20 ± 2 s).

The temporal change in EEG spectral power has been analyzed during NH exposure and recovery. Again, these results have been obtained by the comparison between the Hypoxia-Air group and the Hypoxia-O_2 group. During NH exposure, no statistically significant change (from the baseline) in the EEG spectral power was observed in any group. At the beginning of NH recovery, we observed a transient significant increase in slow-wave activity in both groups. During this transitory period, the relative power in the delta band was almost multiplied by two in the Hypoxia-Air group [from 15.35 ± 2.38% at the baseline to 27.07 ± 2.87% at its highest; $F(18,378) = 1.810$; $P = 0.023$] and in the Hypoxia-O_2 group [from 16.78 ± 2.69% at the baseline to 26.66 ± 2.35%; $F(18,343) = 1.834$; $P = 0.021$]. Almost

Table I. Main PASAT Scores During the Phases of NH Exposure and Recovery in the Normoxia-Air and Hypoxia-Air Groups.

PARAMETERS	NORMOXIA-AIR GROUP	HYPOXIA-AIR GROUP	*F*(1,43)	*P*
Control phase				
Number of responses	6.0 ± 0.0	6.0 ± 0.0	<0.001	1
Percentage of correct responses (%)	92.7 ± 3.1	92.4 ± 3.0	0.007	0.933
Percentage of omissions (%)	1.4 ± 1.0	1.5 ± 1.0	0.001	0.971
Percentage of miscalculations (%)	3.6 ± 1.8	6.1 ± 2.3	0.939	0.338
Percentage of suppression failures (%)	2.2 ± 1.6	0.0 ± 0.0	2.021	0.162
Desaturation-delay phase				
Number of responses	9.0 ± 0.0	8.7 ± 0.6	0.002	0.966
Percentage of correct responses (%)	89.4 ± 2.6	94.3 ± 2.1	1.611	0.211
Percentage of omissions (%)	1.9 ± 0.9	1.6 ± 1.1	0.040	0.843
Percentage of miscalculations (%)	6.3 ± 1.8	2.5 ± 1.4	2.229	0.143
Percentage of suppression failures (%)	2.4 ± 1.2	1.6 ± 0.9	0.119	0.732
Desaturation phase				
Number of responses	32.0 ± 0.0	32.4 ± 1.4	0.138	0.712
Percentage of correct responses (%)	88.2 ± 2.1	70.3 ± 3.9	17.847	<0.001
Delay between errors (s)	23 ± 3	12 ± 1	7.101	0.011
Percentage of omissions (%)	2.6 ± 0.8	9.2 ± 2.4	12.626	<0.001
Percentage of miscalculations (%)	6.5 ± 1.3	16.4 ± 2.3	12.862	<0.001
Percentage of suppression failures (%)	2.7 ± 0.8	4.1 ± 1.0	1.040	0.313
Recovery-delay + recovery phases				
Number of responses	30.0 ± 0.0	28.5 ± 3.0	0.069	0.794
Percentage of correct responses (%)	88.8 ± 2.2	80.0 ± 2.8	5.349	0.026
Delay between errors (s)	23 ± 3	17 ± 3	0.561	0.458
Percentage of omissions (%)	2.0 ± 0.7	5.7 ± 1.8	4.496	0.040
Percentage of miscalculations (%)	6.5 ± 1.2	11.5 ± 2.1	3.947	0.053
Percentage of suppression failures (%)	2.6 ± 0.8	2.8 ± 0.8	0.079	0.785
Stabilization phase				
Number of responses	27.0 ± 0.0	30.1 ± 2.9	0.063	0.801
Percentage of correct responses (%)	88.2 ± 3.0	91.4 ± 2.6	0.612	0.439
Percentage of omissions (%)	3.2 ± 1.2	1.4 ± 0.6	0.948	0.336
Percentage of miscalculations (%)	6.1 ± 1.4	5.3 ± 2.0	0.100	0.754
Percentage of suppression failures (%)	2.4 ± 0.9	1.9 ± 0.8	0.043	0.834

simultaneously, theta activity was constant in the Hypoxia-Air group and moderately increased in the Hypoxia-O_2 group [from 22.70 ± 1.47% at the baseline to 33.02 ± 4.09%; $F(18,343) = 1.802$; $P = 0.024$]. Between-group comparisons showed that the relative power in the delta band was significantly higher in the Hypoxia-O_2 group during the first 16 s of recovery [$F(1,40) = 5.110$; $P = 0.029$], while theta power remained higher during the first 32 s of recovery [$F(1,40) = 5.423$; $P = 0.025$] (**Fig. 3**). Moreover, NH exposure was associated with a significant increase in the 95th percentile spectral edge frequency (SEF95) in both groups. SEF95 significantly increased during the first 112 s of NH exposure in the Hypoxia-Air group [from 25.20 ± 0.50% at the baseline to 27.14 ± 0.40 at its highest; $F(18,378) = 1.692$; $P = 0.038$] and in the Hypoxia-O_2 group [from 25.34 ± 0.47% at the baseline to 26.47 ± 0.35%; $F(18,343) = 1.635$; $P = 0.049$]. During NH recovery, there was a further transient rise in SEF95 in the Hypoxia-Air group. Conversely, in the Hypoxia-O_2 group, NH recovery was associated with a significant decrease in SEF95 during the first 32 s [22.51 ± 1.16% at its lowest; $F(18,343) = 1.847$; $P = 0.019$]. Between-group comparisons showed that SEF95 was significantly lower in the Hypoxia-Oxygen group during the first 32 s [$F(1,40) = 15.254$; $P < 0.001$)] of recovery and from 64 to 80 s [$F(1,40) = 6.304$; $P = 0.016$] (Fig. 3).

Finally, the temporal change in PASAT performance was also analyzed during NH exposure and recovery. The mean percentage of correct responses in the PASAT over time is shown in **Fig. 4**. There was no difference between the Hypoxia-Air group and the Hypoxia-O_2 group in the PASAT during NH exposure. During NH recovery, no difference was shown during the first 32 s. From 32 to 48 s, the percentage of correct responses became significantly lower in the Hypoxia-O_2 group [78.7 ± 5.8% vs. 94.3 ± 2.8%; $F(1,40) = 5.650$; $P = 0.022$]. In parallel, the percentage of omissions was significantly higher in the Hypoxia- O_2 group [6.2 ± 1.4% vs. 0.0 ± 0.0%; $F(1,40) = 5.112$; $P = 0.029$]. Indeed, no omission was made in the group exposed to air. From 48 to 80 s, there was no between-group difference in the percentage of correct responses. However, the Hypoxia-O_2 group showed a higher omission rate [6.2 ± 1.4% vs. 0.0 ± 0.0%; $F(1,40) = 5.112$; $P = 0.029$] since no subject from the Hypoxia-Air group made any omission. The analysis of error patterns show that this absence of omission led to the highest proportion of miscalculations in the Hypoxia-Air group [75.0 ± 13.1% vs. 41.0 ± 14.8%, $F(1,40) = 4.429$; $P = 0.042$] from 48 to 64 s. Finally, there was no difference between the Hypoxia-Air group and the Hypoxia-O_2 group at the PASAT from 80 s.

DISCUSSION

The main aims of this study were to investigate the effects of acute NH exposure on physiological variables and working memory and to investigate the physiological and cognitive effects of oxygen breathing before and after acute NH exposure. Our results clearly illustrate the similar effects of acute NH and acute HH exposures on working memory performance. We also showed that oxygen breathing after acute NH exposure could be deleterious for working memory recovery. These findings are crucial for aeromedical training and may lead to practical recommendations for hypoxia awareness training in NH.

Our results allow us to show for the first time the "pure" O_2-effect-free systemic physiological response to acute severe NH. From the start of 6% O_2 breathing, S_pO_2 remained stable during

Table II. Main Systemic Physiological Measurements During the Phases of NH Exposure and Recovery in the Hypoxia-Air and Hypoxia-O_2 Groups.

PARAMETERS	HYPOXIA-AIR GROUP	HYPOXIA-O_2 GROUP	*F*(1,40)	*P*
Control phase				
mean S_pO_2 (%)	98.7 ± 0.2	99.9 ± 0.1	13.981	<0.001
mean HR (bpm)	85 ± 3	84 ± 4	0.003	0.963
HR variation (bpm)	19 ± 4	21 ± 3	0.003	0.957
Desaturation-delay phase				
mean S_pO_2 (%)	99.1 ± 0.2	99.9 ± 0.1	14.012	<0.001
mean HR (bpm)	93 ± 3	93 ± 4	0.006	0.940
HR variation (bpm)	-2 ± 2	-6 ± 2	1.820	0.185
Desaturation phase				
mean S_pO_2 (%)	76.0 ± 0.8	77.1 ± 0.6	2.066	0.158
mean HR (bpm)	102 ± 4	102 ± 4	0.001	0.998
S_pO_2 variation (%)	-35.3 ± 1.0	-33.9 ± 1.0	0.989	0.326
HR variation (bpm)	18 ± 2	21 ± 3	0.772	0.385
Recovery-delay phase				
mean S_pO_2 (%)	60.3 ± 1.0	62.5 ± 1.1	2.330	0.135
mean HR (bpm)	107 ± 3	110 ± 4	0.316	0.577
S_pO_2 variation (%)	-1.6 ± 0.6	-0.8 ± 0.4	0.326	0.571
HR variation (bpm)	-9 ± 4	-10 ± 3	0.126	0.724
min S_pO_2 (%)	58.5 ± 1.1	60.9 ± 1.1	2.296	0.138
Recovery phase				
mean S_pO_2 (%)	92.7 ± 0.5	82.4 ± 0.9	12.782	<0.001
mean HR (bpm)	89 ± 3	98 ± 5	2.726	0.107
S_pO_2 variation (%)	38.2 ± 1.1	36.7 ± 1.1	0.934	0.340
HR variation (bpm)	-18 ± 4	-5 ± 2	11.001	0.002
Stabilization phase				
mean S_pO_2 (%)	99.1 ± 0.2	100.0 ± 0.1	13.542	<0.001
mean HR (bpm)	82 ± 3	90 ± 4	0.805	0.375

about 36 s (desaturation-delay phase), which may correspond to the emptying of oxygen stores in the lungs and blood. The mean TUC (i.e., the mean duration of hypoxic mixture breathing) lasted 163 ± 3 s. As far as we know, this is the first evidence of the mean TUC value in NH conditions without preoxygenation. As expected, the decrease in S_pO_2 was associated with an increase in HR (classical cardiovascular response to acute hypoxia). At the end of 6% O_2 breathing, S_pO_2 continued to drop during about 20 s (recovery-delay phase), which was associated with a decrease in HR. This delay may rely on both the progressive air arrival into the mask and oxygen transport into the blood. From the start of the S_pO_2 increase, S_pO_2 remained lower in the hypoxic group for 100 ± 13 s (recovery phase). This delay may be related to the time needed to saturate hemoglobin with oxygen molecules. As expected, the increase in S_pO_2, ending the "hypoxic stress," was associated with a decrease in HR (the end of the cardiovascular response to hypoxia).

It is noteworthy that we showed a strong increase in HR (about 20 bpm) in all the groups during the control phase, which can be attributed to the PASAT-induced acute stress effect.[26] Although PASAT is known to evoke acute stress, it is interesting to notice that the PASAT-induced tachycardia is similar to the one evoked by an acute NH exposure (+18 bpm).

Surprisingly, we did not find the generally observed hypoxia-associated slowing of EEG activity[3,19,23] and even found an increase in SEF95, which implies a rise in fast-wave activity. This counter-intuitive observation may be explained by the fact that subjects were cognitively active during NH exposure. Indeed, the PASAT is known to induce a rise in activity in several cortical areas, including the prefrontal cortex.[1] Moreover, the high between-subjects variability in EEG measurements may be involved in the lack of significant change. If this effect is confirmed in future studies, it could become a crucial point for the management of in-flight hypoxic accidents by air traffic controllers. Keeping the "hypoxic" pilot cognitively active may prevent cerebral slowing.

Our results show that acute NH exposure results in a decreased working memory performance. No between-group difference on working memory performance was noticed during phases where S_pO_2 was stable (control phase, desaturation-delay phase, and stabilization phase), showing the relationship between S_pO_2 measurement and cognitive function. During the desaturation phase, while S_pO_2 became lower in the hypoxic group, the percentage of correct responses became lower. During this phase, errors were almost twice as frequent in the hypoxic group. Subjects from this group made both more omissions and miscalculations. During the desaturation-delay and desaturation phases, as S_pO_2 remained lower in the hypoxic group, the percentage of correct responses also remained lower. The delay between errors became equivalent between groups, but subjects from the hypoxic group still made more omissions.

Interestingly, we replicated here the specific effect of hypoxia on miscalculation and omission rates—without change in the suppression failure rate—that we evidenced in HH.[14] This finding confirms the reproducibility and repeatability of the PASAT during both HH and NH exposure and substantiates its advantage for hypoxia awareness training. It is assumed that PASAT performance mainly relies on attentional processes.[28] Therefore, our results confirm the assumption of Fowler et al.,[8] who attributed the working memory deficit induced by acute NH exposure to a (direct or indirect) slowing of the central executive, i.e., the attentional-controlling component of working memory.

The systemic physiological response to acute NH we found here is quite different from the one we previously found in HH. The mean TUC of the Hypoxia-O_2 group (230 ± 5 s) is almost 50% longer than the mean TUC we previously found in HH conditions after a 45-min preoxygenation period (156 ± 7 s). One plausible explanation for that difference is that acute NH, by inducing a higher alveolar-arterial Po_2 gradient, allows more efficient pulmonary gas exchange. During the desaturation-delay phase, contrary to acute HH exposure, acute NH did not induce a rise in HR. This may be due to the fact that subjects were not aware of the change of breathing mixture. Moreover, HH exposure is likely to be more stressful than NH exposure as it is performed in an enclosed chamber. Nevertheless, the minimum S_pO_2 value was very close in the Hypoxia-O_2 group (60.9 ± 1.1%) and in the previous acute HH study[14] (60.1 ± 1.1%). These findings are in line with other works showing a distinct systemic physiological response to NH and HH.[22,24]

Our results show that, although the systemic physiological response to hypoxia is different in NH and HH conditions, working memory is similarly, and reversibly, impaired. We

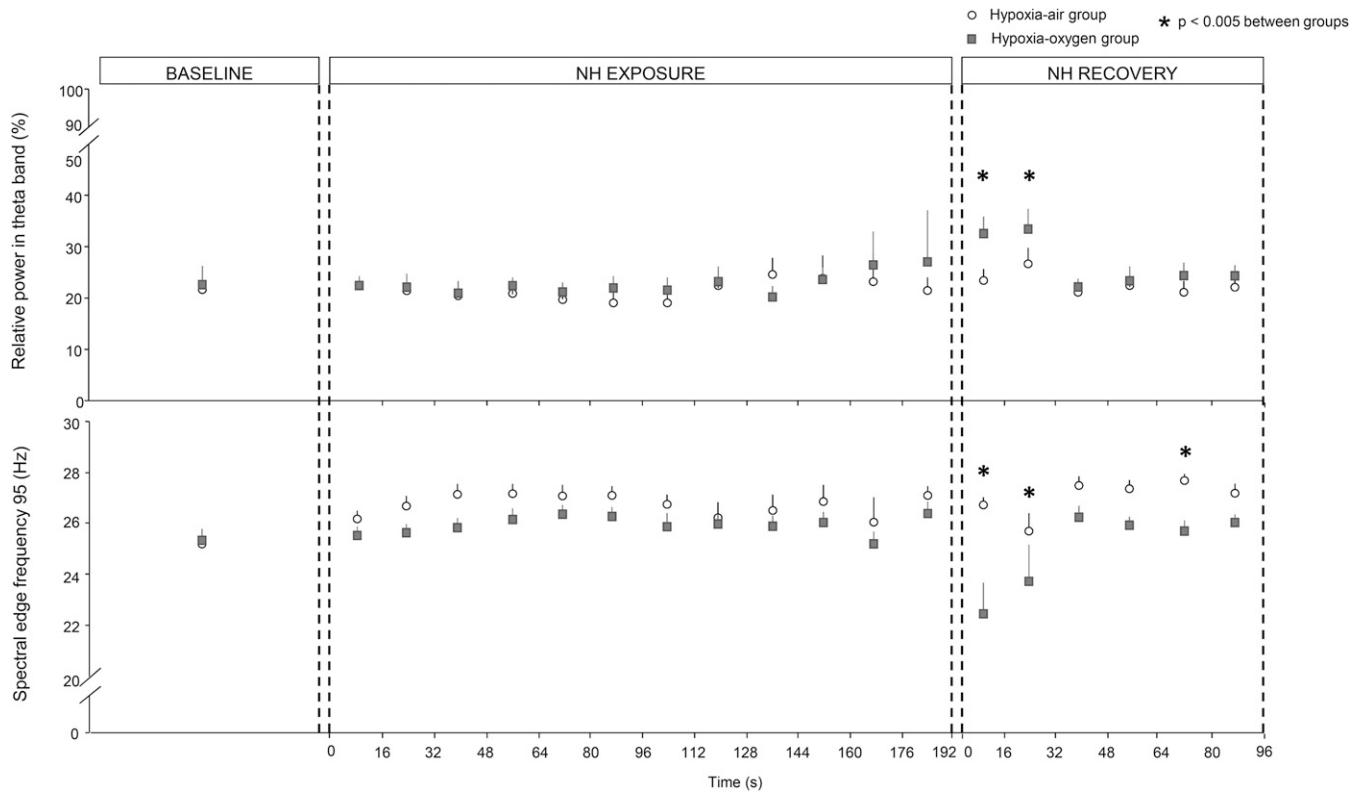

Fig. 3. Mean relative power in the theta band (%; top graph) and the mean 95th percentile spectral edge frequency (Hz; bottom graph) over time (s) during NH exposure and recovery in the Hypoxia-Air (white circles) and Hypoxia-O_2 groups (gray squares). Bars pointing upward represent SEM.

found a strong decrease in the percentage of correct responses in both NH and HH. We also reported an increase in the percentages of omissions and miscalculations without change in the percentage of suppression failure in both environments. Finally, the delay between errors was decreased in both NH (almost 2 times lower) and HH (almost 2.5 times lower). This finding might mean that both NH and HH elicit the same adaptive brain mechanisms. One of these mechanisms may be a transient brain blood flow redistribution to preserve oxygenation of vital areas at the expense of nonvital ones, such as memory-associated structures (e.g., the prefrontal cortex and the hippocampus).[20] Further studies using neuroimaging techniques, such as functional near-infrared spectroscopy (fNIRS) or functional magnetic resonance imaging (fMRI), are needed to confirm this hypothesis.

This study also answered the methodological question of the usefulness of oxygen breathing during NH training. Our results indicate that preoxygenation has no strong effect on how S_pO_2 and HR measurements evolve during acute NH exposure. The more important effect is a lengthening of the delay before desaturation occurs (which may have caused the higher mean S_pO_2 in the Hypoxia-O_2 group during this phase). Similarly, oxygen breathing after acute NH exposure allows a more rapid return to a normal S_pO_2 value (which may also explain the lower mean S_pO_2 in the Hypoxia-O_2 group during this phase). However, acute NH recovery with 100% oxygen was associated with a robust EEG slowing, demonstrated through a significant increase in theta activity and a significant decrease in SEF95. This result suggests that a hypoxia-hyperoxia sequence may be more harmful for the brain than hypoxia by itself. Although unlikely, we cannot reject the hypothesis of a delayed consequence of the preoxygenation period that would show itself during NH recovery. Taken together, these results indicate that 100% oxygen breathing could be deleterious for hypoxic recovery. Unfortunately, we were unable to show a causality effect between the rise in cerebral slow-wave activity and working memory impairment.

The deleterious effect of postresuscitation hyperoxia is a highly topical issue, particularly in the case of cardiac arrest management. Several animal studies show that hyperoxia damages neurons previously exposed to ischemia and exacerbates neuronal injury,[6] leading to behavioral deficits.[11] Inflammation and oxidative stress are likely to be involved in this mechanism.[21] The particularly high vulnerability of the hippocampus, a cerebral structure involved in memory functions (for a review see Yonelinas[29]), to hyperoxic perfusion following ischemia[11] may explain the worsening of memory performance during acute NH recovery with 100% oxygen. Altogether, these findings highlight the importance of EEG monitoring during hypoxia awareness training, since it can provide several accurate indices of cerebral impairment. Long-term cerebral effects of acute hypoxia exposure must also be considered and investigated in the future.

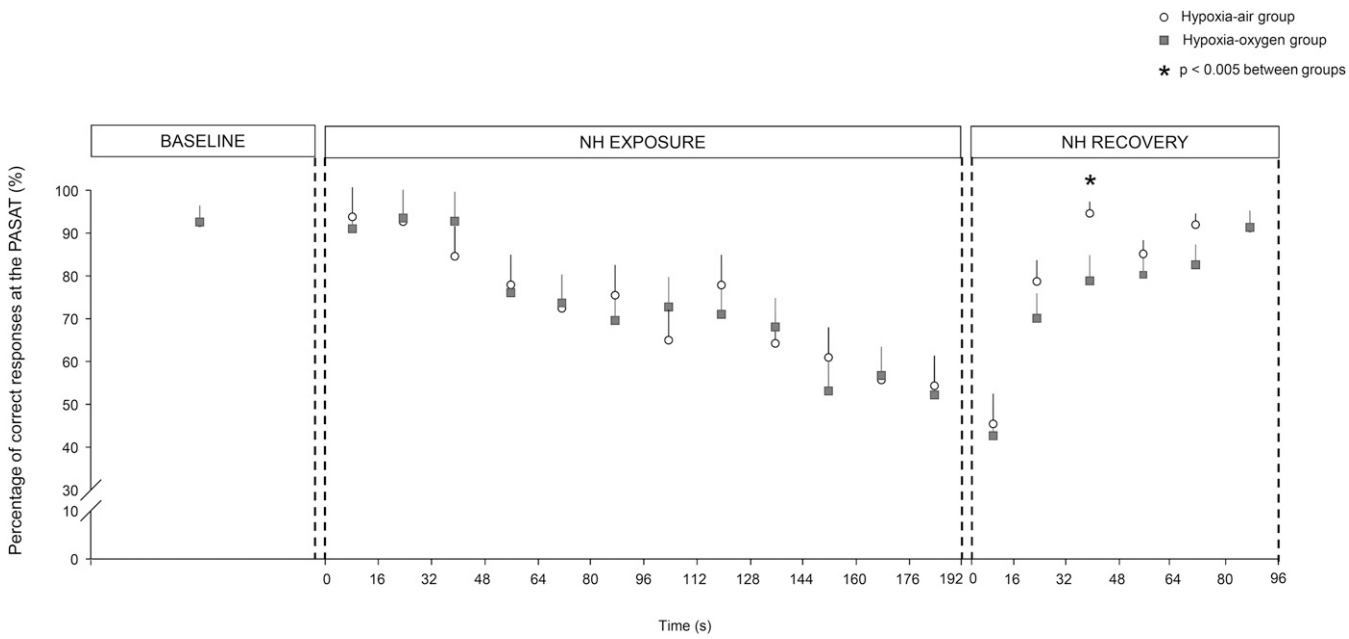

Fig. 4. Mean percentage of correct responses (%) in the PASAT over time (s) during NH exposure and recovery in the Hypoxia-Air (white circles) and Hypoxia-O_2 (gray squares) groups. Bars pointing upward represent SEM.

The deleterious effect of hyperoxia following acute hypoxia exposure is likely to be less important with a lower F_IO_2 level. Yet, according to D'Agostino et al.,[4] cell death in the CA1 region of the hippocampus is significantly less over a 4-h period with a F_IO_2 of 60% than with a F_IO_2 of 95%. The appropriate F_IO_2 level to administer after acute hypoxia exposure needs further investigations.

The major limitation of this study is that we did not directly compare NH and HH exposures, making us unable to find a definitive solution to the debate about the interchangeability of NH and HH in terms of physiological and cognitive responses. Moreover, EEG recordings would have greatly benefited from an event-related potential (ERP) experimental design, which would have permitted strong correlations between EEG activity and working memory performance.

Last, we think that hypoxia awareness training remains crucial for military aircrews and that alternatives to the hypobaric chamber have to be developed. Normobaric devices may represent a good surrogate for a hypobaric chamber as long as some practical recommendations are followed. First, preoxygenation before acute NH exposure is optional as it only lengthens the delay before desaturation. A 3-min preoxygenation period is optimal to fill the lungs with oxygen. Second, in the absence of a medical reason and further studies on the appropriate F_IO_2 level to administer, air rather than 100% oxygen should be preferred for acute NH recovery. A few second delay (about 20 s in our study) between air restoration and S_pO_2 increase is to be expected. Finally, both NH and HH awareness training should incorporate strong physiological monitoring, including EEG recording, and a validated cognitive task, like the PASAT. Because working memory is similarly impaired in both the NH and HH environments, NH seems to be relevant for the refreshment of hypoxia awareness training in experienced aircrews. However, the distinct physiological response to NH and HH, particularly the shorter TUC, justifies the maintenance of HH exposure for initial hypoxia awareness training. As initial training is aimed to draw trainees' attention to the threat represented by in-flight hypoxia and the importance of rapidly recognizing the symptoms and reacting, we think it crucial that their first experience is the closest possible to reality.

In conclusion, this study showed that acute NH caused a strong impairment in working memory that was worsened by oxygen breathing following NH exposure. Our findings also pointed to the physiological response to acute NH being slightly different from the one elicited by acute HH and that oxygen breathing after NH exposure is responsible for a slowing in cerebral activity.

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