Dry-EEG Manifestations of Acute and Insidious Hypoxia During Simulated Flight

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INTRODUCTION: Recently, portable dry electroencephalographs (dry-EEGs) have indexed cognitive workload, fatigue, and drowsiness in operational environments. Using this technology this project assessed whether significant changes in brainwave frequency power occurred in response to hypoxic exposures as experienced in military aviation.

METHODS: There were 60 (30 women, 30 men) student Naval Aviators or Flight Officers who were exposed to an intense (acute) high-altitude (25,000 ft) normobaric hypoxic exposure, and 20 min later, more gradual (insidious) normobaric hypoxic exposure up to 20,000 ft while flying a fixed-wing flight simulation and monitored with a dry-EEG system. Using MATLAB, EEG frequencies and power were quantified and analyzed. Cognitive performance was also assessed with a cognitive task validated under hypoxia. Normobaric hypoxia and O_2 saturation (Spo_2) were produced and monitored using the Reduced Oxygen Breathing Device (ROBD2).

RESULTS: Significant Spo_2 decreases were recorded at acute 25K and insidious 20K simulated altitudes. Significant power decreases were recorded in all frequencies (alpha, beta, gamma, and theta) and all channels with acute 25K exposures. Gamma, beta, and theta frequency power were significantly decreased with insidious 20K exposures at most of the channels. The frequency power decreases corresponded to significant decreases in cognitive performance and flight performance. Most importantly, frequency power suppressions occurred despite 42% of the volunteers not perceiving they were hypoxic in the acute phase, nor 20% in the insidious phase.

DISCUSSION: Results suggest EEG suppression during acute/insidious hypoxia can index performance decrements. These findings have promising implications in the development of biosensors that mitigate potential in-flight hypoxic physiological episodes.

KEYWORDS: brainwaves, hypoxia, physiological episodes.

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We currently have systems to continuously monitor over a thousand mechanical components and systems in our modern aircraft; yet we currently do not continuously monitor the most important system: the pilot. The lack of in-flight physiological data on pilots has become an increasing concern in light of an upward trend in physiological hazard reports from several high-performance aircraft platforms which use onboard oxygen generating systems. With regard to the U.S. Navy, fiscal year 2010 saw roughly 12 physiological events (PE) per 100,000 flight hours reported in the F/A-18AD, followed by a steady upward trend to the most recent year of data, 2017, with over 101 PE/100,000 flight hours.[30] These events are not unique to the F/A-18, as this trend has been a multiservice, multiplatform phenomena, affecting F-35, T-45, EA-6Bs, and T-6 platforms as well.[3] The higher numbers of reported physiological events can be attributed, to some extent, to an increase in aircrew awareness and reporting. The uncertainty around the nature and cause of these events points directly to the need for objective physiological data from the pilot to substantiate and more fully characterize them.

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Wearable biosensors and accelerometers have become ubiquitous in the field of exercise physiology and are fast becoming a tool to monitor workplace productivity.[31] They have been used in a number of operational scenarios such as high-altitude sky-diving,[7] mountain climbing,[33] and centrifuge studies.[8] Despite these recent advanced technological applications of monitoring in extreme environments, current literature regarding the acquisition and monitoring of physiological information during high-performance jet aircraft flight is lacking. Kobayashi documented the efficacy of monitoring cerebral oxygenation during aircraft combat maneuvers using near infrared spectroscopy.[15] Unfortunately, no further studies acquiring normative data using this technology have been published. Recently, Rice et al. validated the use of wearable accelerometers during high performance jet aircraft maneuvers.[26] In addition to accelerometry data, these wearable biosensors acquired basic physiological monitoring such as heart rate, respiratory rate, and temperature.[27] Although these data have been useful in assessing normal and adaptive responses to high accelerative forces, their utility in categorizing physiological events which result in cognitive decrements is questionable. In the best of scenarios, a higher than normal heart rate (tachycardia) or lower oxygen concentration detected by wearable biosensors may suggest hypoxia, hyperventilation, gravity-induced loss of consciousness, or contaminants, but would not necessarily be indicative of cognitive impairment. Therefore, real-time evaluation of cognitive performance in extreme environments is imperative for the identification of physiological hazards which may impair a pilot's ability to fly safely. One way to do this is through real-time monitoring of brain functioning to identify prognostic changes in electroencephalography (EEG) that indicate impaired cognition.

Wet multichannel EEG, which relies on a direct electrical contact with the scalp and requires both skin preparation and the use of a moist electrolyte (i.e., a conductive solution, paste, or gel) has been used in a limited capacity to evaluate brain activity in extreme environments such as hypobaric hypoxia,[16,22,23] hyperventilation,[24] and simulated excessive acceleration,[35] revealing unique wave form patterns suggestive of cognitive decrements. The cumbersome nature of traditional multichannel wet-EEG and their susceptibility to noise has prevented their transition to operationally relevant environments and subsequent utilization as a potential mitigation tool. The recent validation of dry-EEG[19,29,36] as a reliable surrogate means of evaluating cognitive activity (without the need for skin preparation or application of electrolyte) opens the door for evaluating these systems as viable devices for identifying cognitive performance decrements in operationally relevant environments such as high altitude or excessive acceleration.

The primary objective of this study was to evaluate the ability of dry-EEG to identify patterns of brain activity that correlate with cognitive performance decrements and simulated flight performance errors due to various levels of hypoxia. A secondary objective included determining if patterns of brain activity as measured by a dry-EEG system can identify cognitive states that are indicative of cognitive decrements incurred from hypoxia prior to the subjective awareness of hypoxic symptoms. A tertiary objective included evaluating gender differences in hypoxia tolerance and cognitive performance from baseline (sea level) to simulated altitude. Gender differences in brainwave frequency power and cognitive performance at simulated altitude will be described in a companion publication [Gender difference in dry-EEG manifestations of acute and insidious hypoxia. Aerosp Med Hum Perform. 2019; 90. In press.]. Physiological data such as pulse oximetry (PulseOx) for percent oxygenated hemoglobin (S_pO_2) and heart rate were also logged across various levels of normobaric hypoxia and cognitive workloads to further elucidate the normative physiological data across extreme conditions.

METHODS

Subjects

Participants were 60 ($N = 30$ men, 30 women) aeromedically cleared student Naval Aviators, student Naval Flight Officers, and student Naval Flight Surgeons awaiting training or instructing at Naval Air Station Pensacola, FL. The study protocols and procedures were approved by the Navy Medicine Operational Training Center's Scientific Ethical Review Committee under protocol number NMOTC2016.0016, and subsequently approved by a higher level Institutional Review Board at the Naval Medical Research Unit-Dayton, Wright Patterson Air Force Base, OH. After reviewing the protocol, each participant provided informed consent to a project associate investigator who was neither in the participant's chain of command nor involved with his or her medical evaluation. Demographic information such as rank, age, gender, flight hours, and prior history of traumatic brain injury were obtained. Subjects were excluded from the study if they did not have a valid, aeromedical clearance known as an "up chit" or if they had over 25% of their physiological and cognitive performance data deemed as incomplete, a threshold by which it was assumed not valid to impute.

Equipment

Participants were fitted with a standard Naval aviation mask (MBU-23) which interfaces with the reduced oxygen breathing device 2 (ROBD2, Model 6202, Environics®, Inc., Tolland, CT). The ROBD2 is a normobaric, hypoxia-producing device that works by altering the concentration of oxygen an individual breathes with inert nitrogen gas. Through validated algorithms, the device simulates altitude by providing precise concentrations of oxygen and nitrogen via mass flow-controlled sensors.[1] The device is calibrated daily and used to train thousands of aircrews each year regarding the deleterious effect of hypoxia without the side effects of reduced pressure.

Once appropriately fitted with their aviation mask and connected to the ROBD2, breathing sea-level equivalent oxygen, they were connected to oxygen saturation sensors on the finger and forehead, then to the DSI-7 System by Wearable Sensing®

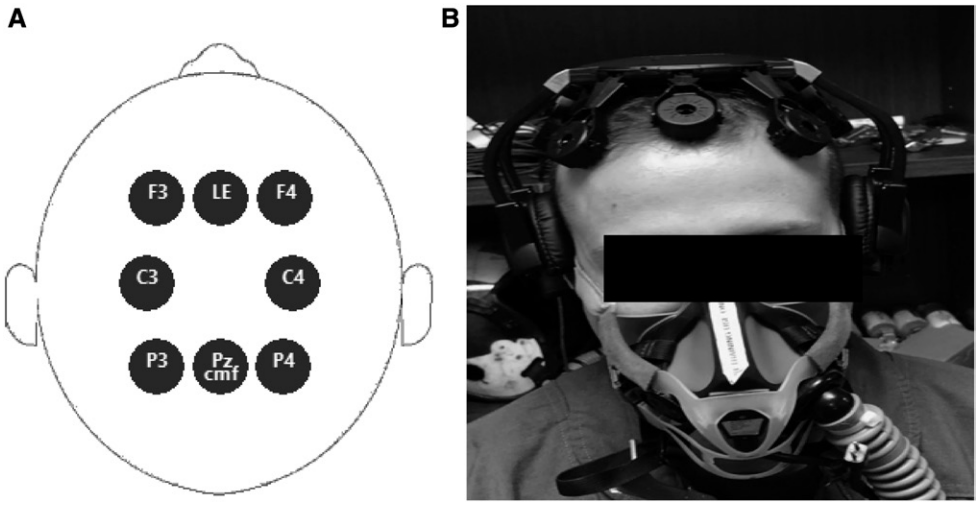

Fig. 1. Schematic channel array of DSI97 & actual photograph of system with O_2 mask. F denotes frontal electroencephalogram channels, C denotes central electroencephalogram channels, P denotes parietal channels, Pz is the parietal midline, and LE the prefrontal mid-line.

(DSI-7, Wearable Sensing, LLC, San Diego, CA). This is a 7-channel, dry-electroencephalogram which uses sensors located within the International 10/20 electrode placement system locations: F3, F4, C3, C4, P3, P4, and linked ears (**Fig. 1**).[12,34] The DSI-7 has a sampling rate of 300 Hz and a band pass rate between 1–50 Hz. The device uses software that classifies various cognitive states and has been validated for mental engagement, workload, and fatigue.[9,20] Although the DSI-7 has the ability to measure cognitive workload by using its proprietary software "Q-states," we independently evaluated each channel frequency and magnitude during various normobaric hypoxic exposures via MATLAB (The Mathworks, Natick, MA)[32] using Fast Fourier transformation (FFT).

Appropriately connected to the ROBD2, oxygen saturation monitors, and fitted with the DSI-7 for brainwave monitoring, the participant was acquainted with the X-Plane version 10.5 simulator.[37] X-Plane predicts the performance and handling of most aircraft; it is a great tool for pilots to keep up their skills in a simulator which flies like a real plane, for engineers to predict how a new airplane will fly, and for aviation enthusiasts to explore the world of aircraft flight dynamics. For the purposes of this protocol, subjects "flew" over Montana in a fixed-wing, single-engine Cessna aircraft, and the simulation provided altitudes and heading each second of the flight which were used to determine flight performance and any changes to flight performance while wearing the dry-EEG system.

To assess cognitive performance during baseline and normobaric hypoxic exposures we used a computerized neurocognitive task called Visual Sequence Comparison (VSC). VSC is a subtest of the Cogscreen-Hypoxia Edition that has been validated to be sensitive to the effects of hypoxia at levels above 12,000 ft (3657.6 m).[25] The task challenges the participant to compare two simultaneously presented, alphanumeric sequences, and it specifically measures visual attention, working memory, verbal-sequential processing, and visual-perceptual speed, with a test-retest reliability (stability) of 0.85–0.89.[2,14]

Procedures

Following informed consent, participants were fitted with a standard Naval aviation mask (MBU-23) which interfaces with the ROBD2. During mask fitting, one of the researchers would give the participant a description of the common symptoms of hypoxia and told the participant to pull a "green ring" beside the chair which would signify they recognized these symptoms were occurring to them. In the study the green ring pull was simulated, but in an operational Naval aircraft the green ring represents a functional handle which, when pulled, provides the aviator 100% oxygen. In our study protocol, although participants may have felt they recognized their symptoms, they were allowed to continue with their normobaric hypoxic exposure until their oxygen (O_2) saturation fell below 60%, or until they were not comfortable to continue with the exposure, and at that time were provided 100% oxygen.

Once fitted with their aviation mask and connected to the ROBD2, breathing sea-level equivalent oxygen, they were connected to oxygen saturation sensors on the finger and forehead, then to the DSI-7 System. The subject was then acquainted with the X-Plane version 10.5 simulator. Upon becoming familiar with the operations of the X-Plane simulator, the participant was instructed to start at a heading of 360° and a simulated altitude of 10,000 ft (3048 m) and began a standard rate climb of 250 ft/min (76.2 m/min) and standard rate turn for 180°, then performed a standard rate descent of 250 ft/min and continued their turn back to the original heading of 360°. This maneuver was performed for 3 min at sea level and then performed at a normobaric hypoxic exposure of 25,000 ft (7620 m) for 3 min (i.e., the acute phase in the study). Brainwave frequency power was determined for all frequencies and channels and compared between sea level and 25K. Participants were provided 100% oxygen if their O_2 saturation fell below 60%.

Following this acute 25,000-ft normobaric exposure, the participant was provided 100% oxygen until their O_2 saturation

Table I. Demographic Information.

	WOMEN (30)		MEN (30)	
SUBJECTS	MEAN	SD	MEAN	SD
Age	24	2.5	23.8	1.7
Height	65.6	2.2	70.5	3
Weight	148.5	17.9	177.6	23.2
BMI	24.2	2.5	25.1	2.5
Flight Hours	22.9	52.3	10.0	17.4
Concussion History	No		No	
Tobacco use	No		No	
Rank	29-ENS, 1-LT		30-ENS	

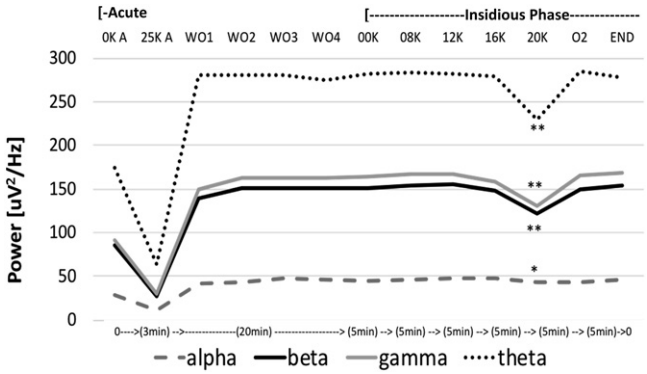

Fig. 2. Grand averages (frequency power) at all frequencies collapsed across all seven channels. Top abscissa represents the acute phase followed by a washout recovery period followed by an insidious (gradual) phase in the normobaric altitude changes in the study; bottom abscissa represents the normobaric exposure timeline of the study. The "0K A", "00K", and "End" were normobaric sea levels with 100% oxygen given immediately after the 20K exposure. The ordinate represents the frequency power.

was greater than 97%, then began a 20-min hypoxia "washout" or recovery period before the next phase of the study. During this washout period they were taught the computerized neurocognitive task called VSC. The participant took this test five times during the washout period to ensure there was no learning effect during the rest of the protocol.

After completion of the hypoxia washout, the participant than began an insidious (i.e., gradual) normobaric hypoxic exposure toward 20,000 ft (20K; 6096 m) simulated altitude. Starting at sea-level equivalent oxygen concentrations, the participant was exposed to 5-min intervals of sea level, 8000 ft (2438.4 m), 12,000 ft (3657.6 m), 16,000 ft (4876.8 m), 20,000 ft, 100% oxygen, and back to sea level, for a total of 35 min. During each 5-min interval, 150 s were spent performing the VSC and the second half was spent performing the simulated flight task of climbing at constant rate and turning for 180° followed by descending at a constant rate and turning to complete a full circle. Outcome measures analyzed at each interval included reaction time, omission errors, and throughput during the VSC, while mean deviation from heading, altitude, and GPS deviations were monitored during the X-Plane simulation. Brainwaves were monitored via the DSI-7 during each interval, and computed frequency magnitude via FFT (3-min windows of frequency power for the acute phase and 5-min windows of frequency power for the insidious phase) and average frequency power was determined at each interval. Oxygen saturations and heart rate were monitored through the 35-min exposure, and study duration runtimes within the 35 min were recorded when the subject pulled the green ring. It was emphasized to the subject throughout the protocol that he/she could discontinue at any time and were immediately provided 100% oxygen if they discontinued or if their O_2 saturation fell below 60%.

Statistical Analysis

Power analysis was performed using differences found in prior studies between men and women exposed to fatigue and ischemia[6] indicating a 35% reduction in reaction time change from baseline to altitude exposure of 20K for women compared to men.[5] For our smallest sample size comparison, assuming there was a 0.5-ms standard deviation within-subject reaction time for subjects, and setting $\alpha = 0.05$ and $\beta = 0.80$, and an estimated effect size of 35%, we estimated approximately 25 subjects per group.

For the EEG analyses, a 7 (electrode channels) × 2 (altitude: sea level vs. 25K or sea level vs. 20K) repeated-measures analyses of variance (ANOVA) was used to assess whether differences existed in brainwave frequency magnitude. For the other variables (S_pO_2, cognitive performance, etc.), repeated measures ANOVAs were performed. For the self-reported hypoxia symptoms, nonparametric McNemar Chi-squared analyses were performed. All statistical analyses were conducted using the Statistical Package for the Social Sciences (SPSS®, IBM Corp., Armonk, NY).[10]

RESULTS

A total of 73 subjects volunteered for this project. There were 13 subjects who were excluded for incomplete S_pO_2 analysis or incomplete cognitive performance results, leaving the total $N = 60$ (30 women, 30 men). No subjects were excluded for incomplete brainwave acquisition. The population was homogenous with no significant differences in average age, body mass index (BMI), or flight hour history (**Table I**).

Table II. Paired Comparisons (Sea Level vs. Altitude) at Each Channel.

ACUTE (SEA LEVEL 00K vs. 25K) PAIRED *t*-TESTS (ALL REPORTED MEAN DIFFERENCES SIGNIFICANT AT $P < 0.0001$)					
FREQUENCY	**CHANNELS WITH SIG. MEAN DIFF.**	**MEAN DIFFERENCES**	***t*(59)**	**COHEN'S *d***	**EFFECT SIZE**
Beta	LE, F3, F4, C3, C4, P3, P4	33.4 to 55.5	11.1 to 16.7	1.4 to 2.2	Large
Alpha	LE, F3, F4, C3, C4, P3, P4	12.7 to 21.0	10.2 to 12.3	1.3 to 1.6	Large
Gamma	LE, F3, F4, C3, C4, P3, P4	2.6 to 7.6	5.4 to 12.6	0.69 to 1.6	Medium-Large
Theta	LE, F3, F4, C3, C4, P3, P4	46.2 to 56.1	7.8 to 13.8	1.0 to 1.8	Large
INSIDIOUS (SEA LEVEL 00K vs. 20K) PAIRED *t*-TESTS (ALL REPORTED MEAN DIFFERENCES SIGNIFICANT AT $P < 0.025$)					
Beta	LE, F3, F4, C3, C4, P3, P4	21.3 to 37.1	4.0 to 6.0	0.57 to 0.77	Medium
Alpha	NS	NS	NS	NS	NS
Gamma	LE, F3, F4, C3, C4, P3, P4	3.2 to 6.6	3.2 to 4.8	0.41 to 0.62	Medium
Theta	LE, F3, F4, C3, C4	16.3 to 29.4	2.2 to 4.2	0.28 to 0.54	Medium

NS: not significant.

Table III. S_pO_2 & HR Paired Comparisons of Sea Level vs. Altitude.

	MEAN DIFFERENCE	SD	*t*(59)	*P*	COHEN'S *d*	EFFECT SIZE
S_pO_2 Paired Comparisons						
Sea Level 0KA vs. 25KA	−14.32	3.09	35.9	0.000	4.63	Large
Seal Level 00K vs. 20K	−22.66	6.52	26.6	0.000	3.43	Large
HR Paired Comparisons						
Sea Level 0KA vs. 25KA	15.7	6.21	19.5	0.000	2.52	Large
Seal Level 00K vs. 20K	18.04	7.9	17.74	0.000	2.29	Large

Fig. 2 illustrates the four frequencies and the impact of hypoxia to these frequencies in the acute 25K and insidious 20K phases of the study. For simplicity, the frequencies illustrated are the grand averages in the FFT power for that frequency collapsed across the seven EEG channels. A series of repeated measures ANOVA were conducted to determine mean difference in power for each frequency across simulated altitudes. In the acute phase, theta, gamma, and beta showed a significant reduction in mean power of approximately 70% from sea level to 25K and approximately 20% from sea level to 20K. Alpha power exhibited a reduction of approximately 60% from sea level to 25K, however, no significant change in power from sea level to 20K.

Table II represents the subsequent paired comparisons performed based upon the significant frequency power decrements noted in Fig. 2. The top portion of Table II represents the analyses of the acute phase and shows that all channels and all frequencies were suppressed significantly when going from sea level to 25K. In the bottom portion of the table representing the analyses of the insidious phase, three of the four frequencies (sans the alpha frequency) were suppressed when going from sea level progressively to 20K, which is clearly illustrated in Fig. 2, but not all channels demonstrated significant suppression.

The S_pO_2 data recorded with the ROBD was analyzed using a repeated measures ANOVA. There was a significant main effect for S_pO_2 [$F_{Greenhouse\text{-}Geisser}$ (1.4, 82.8) = 579.4, P = 0.000, partial eta square = 0.91, large effect size]. Paired comparisons were subsequently performed and are in **Table III**. The S_pO_2 data are illustrated along with the reported suppression of beta power in the acute and insidious phase in **Fig. 3**. The S_pO_2 data parallel the EEG frequency at these altitudes and Table III reflects the significant drop in S_pO_2 when stepping to 25K or 20K altitudes from sea level. Note also in Fig. 3 the number of "green ring" pulls subjects performed in the acute and insidious phase. Of the 60 subjects, 42% did not pull the ring in the acute phase and 20% did not pull it in the insidious phase. Moreover, 10 subjects pulled the green ring during the insidious exposure who were not physiologically hypoxic, making the total percent who either misidentified or failed to recognize they were hypoxic 37%. Heart rate (HR) data was also recorded with the ROBD and was analyzed similarly to the S_pO_2 data using a repeated measures ANOVA. There was a significant main effect for HR [$F_{Greenhouse\text{-}Geisser}$ (2.04, 120.3) = 159.4, P = 0.000, partial eta square = 0.73, large effect size]. As S_pO_2 decreased at 25K and at 20K, HR increased at these altitudes.

X-Plane recorded subjects' GPS "Montana" headings during the simulated flight and the deviations in simulated altitude from the true coordinates. These data were analyzed in a repeated measures ANOVA. (Only 23 subjects' X-Plane data were successfully recorded and available.) The main effect for Altitude (i.e., deviations from altitude) was statistically significant [$F_{Greenhouse\text{-}Geisser}$ (3.9, 86.3) = 3.10, P = 0.02, partial eta squared = 0.12, medium effect size]. **Fig. 4** illustrates X-Plane performance. Note where S_pO_2 and beta power "dip" at 20K, flying performance shows its greatest deviation. This was confirmed statistically in the paired comparisons of mean altitude deviation from assigned constant rate turn and climb while at sea level vs. simulated altitude with normobaric hypoxia of 20K equivalent (see **Table IV**).

A series of repeated measures ANOVAs were used to analyze cognitive performance data from the CogScreen-HE VSC task. **Table V** identifies the significant main effects and differences in reaction time, accuracy, and throughput between simulated altitudes. **Fig. 5** overlays the CogScreen performance data over the beta power and S_pO_2 data. The significant findings in CogScreen performance coincide with the dip in EEG beta power and S_pO_2 which occurred at 20K altitude. Reaction time (RT) was significantly increased, and accuracy and throughput were significantly lower at 20K when compared to sea level. Fig. 5 depicts the significant decreases in accuracy combined with the significant increases in RT occurring at 20K, resulting in a significant decrease in throughput, as measured by accuracy % divided by average RT, which correlates with the observed significant decreases in average beta power.

The subjects' self-reported ratings of hypoxic symptoms were inputted into a nonparametric test for analyses. The

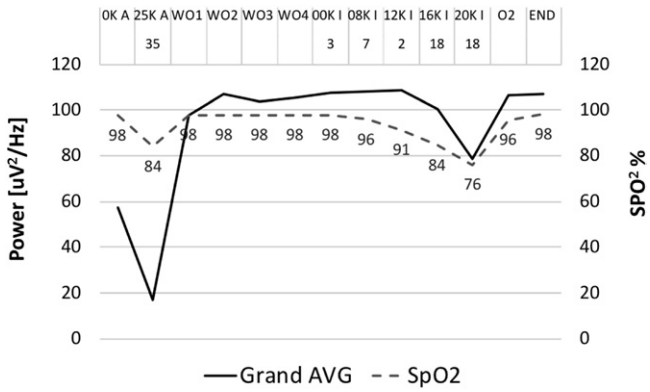

Fig. 3. Grand average beta power, S_pO_2, and number of "green ring" pulls. The superior horizontal axis represents the time line of the protocol in terms of altitude exposures, where 0K and 25K were the acute hypoxia exposure, lasting 3 min for each exposure, followed by a 20-min washout period (WO1, WO2, WO3, WO4), then an insidious hypoxia exposure of 5-min intervals at each altitude. The number under the exposure value represents the number of subjects who pulled the green ring, demonstrating they had recognition of their hypoxia symptoms. For example, the number 35 under 25K represents 35 of our participants recognized their hypoxia symptoms and simulated pulling a green ring (25 of 60; 42% failed to recognize their symptoms despite significant decreases in brainwave power and S_pO_2).

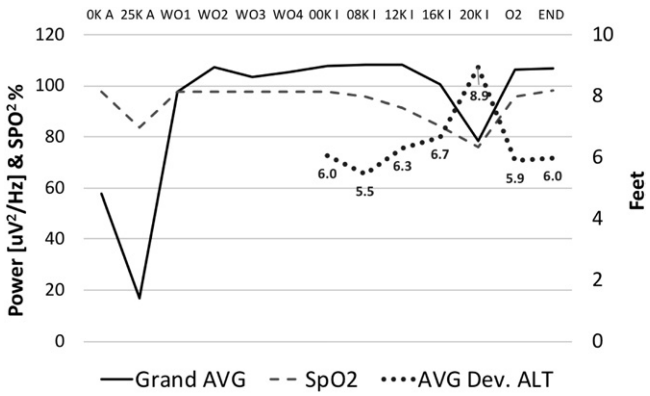

Fig. 4. Grand average beta power, S_pO_2 (left scale), and X-Plane performance, as measured by average deviation from assigned altitude in feet (right scale).

self-reported ratings are illustrated in **Fig. 6**. McNemar's Chi-squared [$\chi^2(1) = 5.06$, $P < 0.024$] showed subjects reporting significantly more experiences of fast heart rate in the 25K acute phase than in the 20K insidious phase. McNemar's Chi-squared [$\chi^2(1) = 8.26$, $P < 0.004$] showed subjects reporting significantly more headaches in the insidious 20K phase than in the 25K acute phase.

DISCUSSION

To our knowledge this is largest study to evaluate brainwave changes to hypoxia and the first to evaluate brainwave changes to hypoxia with dry-EEG technology. Various studies have evaluated brainwave patterns following a transient anoxic event such as cardiopulmonary arrest.[13,28] Their findings are in general consistent with ours in that they have found a suppression of brainwave frequency and voltage following an anoxic/hypoxic event. However, observations from these studies were performed several hours after the anoxic event and are, therefore, less pertinent to the real-time evaluations of brainwaves we have obtained in this study. Continuous wet-EEG monitoring during extreme operational environments using a standard 10/20 wet-EEG system requiring a conductive gel and EEG channels that are susceptible to noise has proven technologically difficult, making appropriate comparison of our findings challenging. Over the last 30 yr, three studies have evaluated wet-EEG techniques in hypobaric environments and we have summarized their findings and compared them to ours in **Table VI**.

Consistent with the findings of Ozaki and Kraaier, we observed significant decreases in alpha frequency power with acute hypoxic exposures to 25K.[16,22] In contrast to these prior studies we did not see suppression of alpha frequency power with insidious hypoxic exposures. Moreover, we found significant suppression in all frequency power with acute exposures to 25K (in metrics, 7620 m) and significant suppression in gamma, beta, and theta following insidious exposure to 20K (6096 m). Additionally, we found a significant increase in frequency power from baseline in all frequencies with return to sea-level conditions following acute 25K exposures. No significant difference from the new baseline was observed following return to sea level following 20K insidious exposures.

A plausible explanation for the differences in our observations with regards to theta, beta, and gamma frequency power may be the spectral power changes may manifest differently across the various channels in the prior reports than the seven channels we used in our study because our channel locations were primarily at the superior aspect of the scalp and further were protected from noise by the electrode design that includes a Faraday-cage housing (i.e., a copper housing) within the individual electrode biosensors. Superior EEG channels (Fig. 1), due to their greater distance from the reference (the ear) channel, tend to increase in maximal power compared to channels close to the ear, which show smaller potential difference.[17,21] Although not having recorded data from the temporal and posterior cortical areas as thoroughly as a standard 10/20 wet EEG represents a limitation of our study, the decision was purposeful in that for future transition of this technology, channels would more likely need to be placed in these superior locations to be compatible with flight helmets.

By measuring brainwave changes in both a traditional fashion via spectral power analysis and a nonlinear evaluation with Approximate Entropy (ApEn),[23] which quantifies the complexity (or irregularity) of a signal and offers robustness for short and noisy data sets,[6] Papadelis et al. found significant decreases in ApEn at 25,000 ft (7620 m), specifically in the frontal and central cerebral cortex. ApEn may represent a more appropriate method of measuring the effects of hypoxia in dynamic analysis of short data sets, a usual characteristic of hypoxic conditions, moreover for data that have been collected in hypobaric chambers where the environment injects significant electrical noise in EEG measurements.[26] In our study, we used linear spectral power analysis from channels which were protected from noise through built-in Faraday housing within each sensor. It is of interest that both methods of analyzing electrical brainwave activity that protected against electrical noise, either through computer algorithms with ApEn or physically with copper Faraday cages, as with our dry-EEG, observed frequency power suppression with acute hypoxia, whereas the earlier studies by

Table IV. Paired Comparisons of Altitude Deviations from Sea Level vs. Altitude.

PAIRED SAMPLES TEST	MEAN DIFFERENCE IN ALTITUDE DEVIATION	SD	*t*(22)	*P* (2-TAILED)	COHEN'S *d*	EFFECT SIZE
00K vs. 8K	0.58	3.80	0.73	0.47	NS	--
00K vs. 12K	−0.25	2.37	-0.50	0.62	NS	--
00K vs. 16K	−0.62	3.74	-0.79	0.44	NS	--
00K vs. 20K	−2.87	3.74	-3.69	0.00	-0.77	Medium
100% O_2 vs. 20K	−3.03	4.79	-3.04	0.01	-0.63	Medium
END vs. 20K	−2.96	3.68	-3.85	0.00	-0.80	Large

NS: not significant.

Table V. Paired Comparisons of Reaction Time (RT), Accuracy, and Throughput from Sea Level (00K) vs. 20K Altitude.

PAIRED SAMPLES TEST	MEAN DIFFERENCE	SD	*t*(59)	*P*	COHEN'S d	EFFECT SIZE
RT 00K vs. 20K	−142.3(ms)	299.05 (ms)	−3.69	0.000	−0.48	Medium
Accuracy 00K vs. 20K	2.60	5.21	3.59	0.001	0.46	Medium
Throughput 00K vs. 20K	3.62	6.44	4.35	0.000	0.56	Medium

Ozaki[22] and Kraier,[16] who did not have either noise reduction software or the miniature Faraday technology surrounding their sensors, found decreases only in alpha and increases in theta and delta. This observation suggests that using noise reduction software and physical barriers, such as the copper housed sensors, will be essential for the consistency and correlations of future investigations evaluating brainwave activity in operational environments.

From a pathophysiological perspective, our findings of power spectral suppression best matched Martin's observation that hypoxia leads to a sequence of alterations in transmembrane electrochemical gradients, and hence in surface EEG patterns, which depends on the duration and severity of energy substrate deprivation.[18] Energy substrate deprivation is a variable condition of brain energy metabolism determined by the dynamic relationship between energy substrate supply (oxygen and glucose) relative to energy substrate demand. Glucose and oxygen are the substrates for oxidative metabolism in the brain under normoxic or normobaric conditions. Under hypoxic conditions the brain may continue to use glucose, but will quickly be depleted of adenosine triphosphate as a result of oxygen deprivation.[11] When the adenosine triphosphate supply eventually declines to levels insufficient to maintain the activity of the ion pumps, rapid and widespread membrane depolarization occurs,[11] causing an extensive depression of synaptic transmission, and therefore electrophysiological functional neuronal isolation, which may account for our observation of global power spectral suppression and Papadelis'[23] approximate entropy decrease during the hypoxia exposures.

Another significant difference from the three prior studies was that our study included a large population of female participants ($N = 30$). Kraaier did not evaluate any women, while Papadelis and Ozaki each only evaluated five.[16,22,23] In contrast, 50% of our data collection occurred from women and there appears to be significant differences in theta and gamma suppression with insidious hypoxic exposures between genders. This novel finding will be described in detail within our companion paper to this article, "Gender difference in dry-EEG manifestations of acute and insidious hypoxia" [Aerosp Med Hum Perform. 2019; 90. In press.].

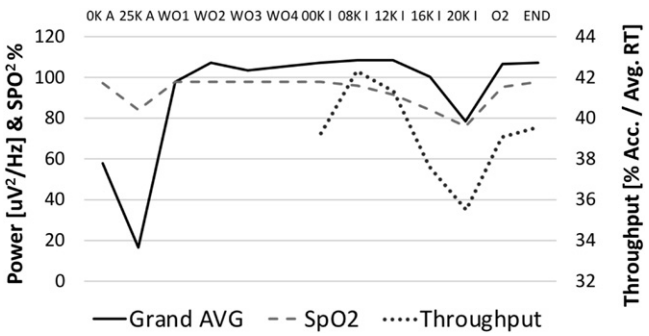

Fig. 5. Grand average beta power, S_pO_2 (left scale), and CogScreen-HE throughput (right axis).

We did exclude 13 participants from this study for incomplete data. This was due to technical errors of recording for S_pO_2 and the cognitive performance test, not because of intersubject variation in hypoxia tolerance or cognitive tests performance. For these excluded subjects the demographics of average age, weight, concussion history, and flight hours were not significantly different. Of the 60 subjects we included in our final population, we only obtained 23 complete flight performance data sets; again, this was due to technical difficulties with proper recording with the X-plane software. Fortunately, despite this lower *N*, we were able to observe differences in flight performance between sea level and 20K exposures, as measured by deviation from assigned altitude while performing a constant rate turn, climb, and descent.

There are several methodological differences between these studies which should be considered prior to drawing final conclusions. The most prominent of these differences would be utilizations of dry- vs. wet-EEG sensor systems. Prior studies have demonstrated a high correlation in brainwave morphology between wet- and dry-EEG under various cognitive tasks.[29,36] Additionally, this technology has advanced since these initial correlations; foremost among these advancements would be the aforementioned Faraday cage housing of each sensor, which decreases the sensors' susceptibility to ambient electrical noise. Moreover, our sampling rate (300 Hz) was over twice that of Ozaki and Kraaier and 40 Hz greater than Papadelis, providing greater temporal resolution of our data.

Our hypoxic exposures were produced by a reduced oxygen breathing device described by Artino.[1] It is unlikely hypoxia-induced brainwave changes produced by gas mixtures would differ from hypobaric hypoxia as both delivery mechanisms produced significant changes in S_pO_2 when measured. Further, delivering hypoxic exposures via an aviation mask, as with the reduced oxygen breathing device, is more realistic with regards to tactical aircraft environments, where pilots are required to wear O_2 masks from takeoff to touchdown.

A limitation of this study was that we did not take into account the other key substrate important for neuronal activity, glucose. Future studies will incorporate a 72-h food log prior to study entry and, further, we will be evaluating various diets and their effect on neuronal activity and response to hypoxia.

It is probable the acute 25K exposure affected the brainwave changes seen during the insidious exposure. All subjects evaluated had significant increases in baseline power frequency following their acute 25K hypoxic exposure. This increase in baseline power, depicted in Fig. 2, is seen throughout the 20-min washout period and suggest a longer recovery time to return to baseline, even when breathing 100% oxygen and

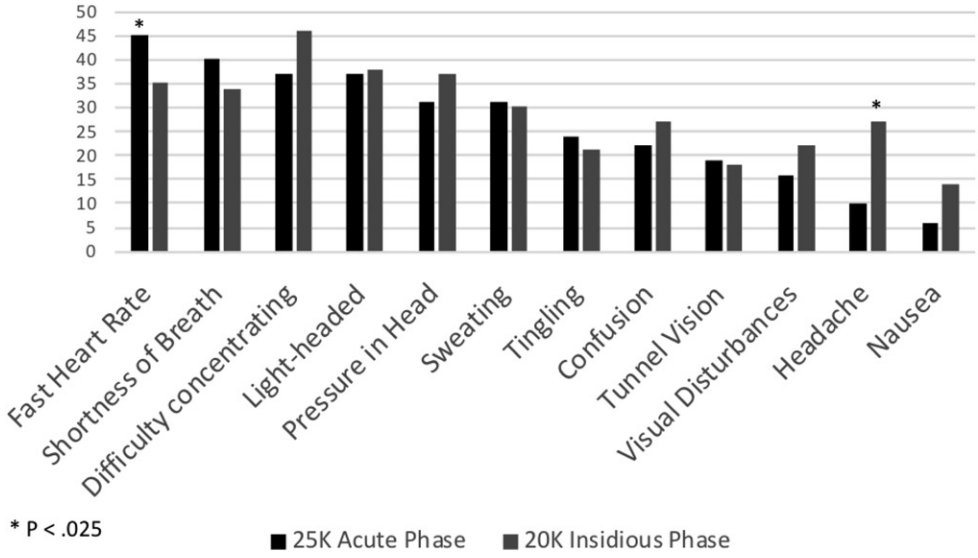

Fig. 6. Subject self-report of hypoxia symptoms.

returning to normal S_pO_2 levels (i.e., > 97%). To better understand this interaction, we plan on randomizing these exposures during our upcoming phase II validation study. We also plan on increasing the length of analysis in future studies to determine when this spike in frequency power observed following hypoxia returns to baseline.

Finally, aviation performance, as measured by deviation from heading and assigned altitude, and cognitive performance as measured by decreases in accuracy, throughput, and increases in reaction time were significant and predictably affected at 20K. These performance changes have been reported previously.[4,25] Observing significant aviation-related cognitive performance changes that correlated well with suppression of gamma, beta, and theta power frequency at 20K, to our knowledge, has not been reported elsewhere, and is of operational importance if we are to use dry-EEG technology as a surrogate monitor for pilot impairment.

Our results suggest that frequency power as measured by common off-the-shelf dry-EEG technology is significantly suppressed with both acute and insidious hypoxic exposures. Suppression of EEG frequency power as measured by this common off-the-shelf dry-EEG corresponds to decrements in S_pO_2, cognitive performance, and simulated flight performance. These results suggest that dry-EEG technology may potentially be useful in identifying PEs. Further validation of this technology with fatigue, acceleration, and hypobaric environments is underway.

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Table VI. Comparison of Methodology and Results of Studies Evaluating Brainwave Changes with Hypoxia.

STUDY	SUBJECTS (W/M)	EXPOSURE/ DURATION	EEG/CHANNELS	SAMPLING RATE/BAND PASS	RESULTS
Rice (2019) (current study)	60 (30/30)	25K, 8K, 12K 16K, & 20K Mixed gas 3 min/5 min	Dry/7 channels of standard 10/20 (copper-housed sensors)	300 Hz	Significant suppression in all frequency power with acute 25K
				1–50 Hz Noise reduction software	Significant suppression of theta, beta, gamma with 20K insidious exposure
Papadelis (2007)[23]	10 (5/5)	25K/20K/15K	Wet, 19 channels of standard 10/20 (noncopper housed sensor)	256 Hz, 0.5–45 Hz	ApEn suppression in all channels
		Hypobaric chamber, 3 min each		Noise reduction software	Transient increase in alpha & theta 25K
Ozaki (1995)[22]	15 (5/10)*	10K, 13K, 16K, 20K	Wet, 16 channels of standard 10/20 (noncopper housed sensor)	128 Hz	Significant decreases in alpha 10K, 13K, 16K, and 20K
		Hypobaric chamber, 25 min each		0.5–60 Hz	Significant increases in theta at 20K
Kraaier (1988)[16]	36 (0/36)**	20K	Wet, 8 channels of standard 10/20 (noncopper housed sensor)	100 Hz	Significant decreases in alpha at 20K, suppression of beta
		Hypobaric chamber, 19 min		0.1–30 Hz	Significant increases in theta & delta at 20K

* Only 5 subjects completed the 25-min exposure to 20K.
** Only 21 subjects completed the 19-min exposure to 20K.

able to be accomplished. The authors' opinions do necessarily reflect the views of the Department of Defense and we do not have any financial relationships to disclose.

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