Electrophysiological correlates of hyperoxia during resting-state EEG in awake human subjects

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Competing Interests: The authors declare no competing financial interests

Keywords: Oxygen, Resting state, EEG, Alpha, hyperoxia

Abstract

Recreational use of concentrated oxygen has increased over the past decade. Claims have been made that hyperoxic breathing can reduce fatigue, increase alertness, and improve attentional capacities; however, few systematic studies of these potential benefits exist. Here we examined the effects of short-term (15 minutes) hyperoxia on resting-states in awake human subjects by measuring spontaneous EEG activity between normoxic and hyperoxic situations, using a within-subjects design for both eyes-opened and eyes-closed conditions. We also measured respiration rate, heart rate, and blood oxygen saturation levels to correlate basic physiological changes due to the hyperoxic challenge with any brain activity changes. Breathing short term 100% oxygen led to expected physiological changes including increased blood-oxygen saturation levels, decreased heart rate, and a slight, non-significant, decrease in breathing rate. Changes of brain activity were apparent, including decreases in low-alpha (7-10 Hz), high-alpha (10-14 Hz), beta (14 – 30 Hz), and gamma (30 – 50 Hz) frequency ranges during eyes-opened hyperoxic conditions. During eyes-closed hyperoxia, increases in the delta (0.5 – 3.5 Hz) and theta (3.5-7 Hz) frequency range were apparent together with decreases in the beta range. Hyperoxia appeared to accentuate the decrease of low alpha and gamma ranges across the eyes-opened and closed conditions suggesting that it modulated brain state itself. As decreased alpha during eyes-opened conditions has been associated with increased attentional processing and selective attention, and increased delta and theta during eyes-closed condition associated with the initiation of sleep, our results suggest a state-specific and perhaps opposing influence of short-term hyperoxia.

Introduction

Over the past decade, recreational use of oxygen (O_2) has increased, and oxygen providers have suggested therapeutic benefits of using O_2 to reduce stress, boost energy, and increase alertness (Bren, 2002), yet, there is little evidence to validate these benefits (Walsh, Thimmesch, D'Achiardi, & Pierce, 2011). Both hypoxic and hyperoxic states can be induced in the body by respectively lowering or raising O_2 content (normally 21%) in inhaled gas. Intermittent exposure to hypoxia has been associated with global impairments in executive functioning (Saunamäki, Himanen, Polo, & Jehkonen, 2009), sleep disturbances (Hamrahi, Stephenson, Mahamed, Liao, & Horner, 2001), cardiovascular and metabolic problems (Marin, Carrizo, Vicente, & Augusti, 2005; Levy et al., 2008) and the induction of neuronal apoptosis (Xu et al., 2004). Given that hypoxia is associated with physiological and brain function impairments, is the opposite true for hyperoxia? That is, are there neural and cognitive benefits of inducing a shorter term (15-minute) hyperoxic state?

In view of the fact that the brain has high metabolic requirements, and since metabolic consumption of the brain appears to correspond very well to the degree of neuronal/functional activation on a moment to moment basis (Raichle & Mintun, 2006; Magistretti & Allaman, 2015), it would stand to reason that providing additional oxidative substrate to the brain might well produce measurable changes in its functional electrophysiological state. Previous work has shown that hyperoxia induces changes in basic physiological parameters such as breathing and heart rate and in turn, these changes may directly or indirectly affect brain state or function (Berssenbrugge et al., 1983; Colrain et al., 1987; Desai et al., 2015; Pack et al., 1992; Tsanov et al. 2014; Yackle et al., 2017). Indeed, hyperoxia is also known to induce changes in cerebral blood flow (Bergo and Tyssebotn 1995; Bew et al., 1994; Busija et al., 1980; Floyd et al., 2003;

Kety and Schmidt, 1948; Lund et al., 1999; Watson et al., 2000). which might also be expected to result in a functional change in the operation of the brain, although while cerebral blood flow and brain state tend to be well correlated, they can show independence from each other (Bangash et al. 2008; Braun et al. 1997; Hajak et al. 1994; Hofle et al. 1997). In our own lab, we have observed that providing 100% oxygen to spontaneously breathing urethane-anesthetized rats with implanted cortical and hippocampal electrodes shifts forebrain state towards more deactivated slow-wave (≤1Hz) patterns (Whitten et al., 2009; Hauer et al, in press). This suggests that ongoing oscillatory EEG measures of brain state may be an effective assay for the effects of hyperoxia and that (perhaps paradoxically) excess oxygen may promote EEG rhythms that are typically associated with decreased levels of arousal. Two human studies appear to indicate this as well. In the first, breathing 100% oxygen appeared to increase coupling in the “default mode network” (Wu et al., 2014), a group of brain regions that show more activation during baseline, sleeping and anesthetized conditions, as compared to instances of alert processing (Raichle, 2015). In the second, increasing inhaled oxygen content to 35% produced an increase in delta (1-4Hz) activity together with a decrease in both beta and gamma power (Seo et al., 2007). In a further independent study, however, no significant EEG changes were observed following a one-hour period of breathing 100% O_2 (Kaskinoro et al., 2008). Croal et al., reported decreases in occipital lobe alpha and beta oscillations using MEG, but without any concomitant changes in cerebral blood flow. In all these studies, behavioural state was not controlled and the influence of physiological variables such as respiratory and heart rate changes were not taken into account. Recently, however, Sheng et al., (2017) reported decreases in EEG alpha activity during sustained-attention and task-evoked conditions, comparing 5-minute interleaved oxygen and control blocks. While this study did control behavioural state under alert, sustained attention

conditions, there has still been no systematic evaluation of the impact of hyperoxia on EEG oscillations in resting-state conditions. However, in all these studies, behavioural state was not controlled and the influence of physiological variables such as respiratory and heart rate changes were not taken into account.

In the present study, we examined the short term (15 minutes) influence of breathing (100% oxygen on resting-state EEG during wakefulness in two simple situations that produce prominent state-dependent differences in the production of alpha activity: eyes-opened and eyes-closed conditions. We ensured that oxygen saturation was maximised during the hyperoxic condition and we also monitored both respiratory and cardiac rhythms during our manipulations. We show that hyperoxia has a state-dependent and complex influence on brain activity that may indicate enhanced attention during eyes-opened conditions while promoting the initiation of sleep when the eyes are closed.

Method

Procedure

Figure 1 illustrates the experimental protocol used. Our experiment consisted of a counterbalanced ABAB design where either 100% oxygen or regular air was provided for ~15m. Within each block, participants completed an alternating eyes-opened/eyes-closed resting-state condition, again in an ABAB design, for 3 minutes each. The eyes-closed condition was always the first and third trial, and the eyes-opened task was always the second and fourth trial. These alternating conditions were chosen based on the reliable observation that power in the alpha band increases when the eyes are closed, and decreases when the eyes are open. Therefore, regardless of what effect hyperoxia is found to have on oscillatory activity, we will be able to show that our conditions can replicate one of the consistent results in the oscillation literature. We collected

data from 26 participants (M = 22.2, range = 18 – 38, 18 female), all of whom were right handed, had normal or corrected vision, and were screened for relevant medical or neurological illnesses. All participants gave informed consent and were compensated for their time. The Health Research Ethics Board at the University of Alberta approved all experimental procedures.

EEG data recording and preprocessing

EEG data was recorded with a 32-channel EEG system inside a radio-frequency attenuated chamber. Standard procedures for reducing electrical impedance between the skin and electrodes were followed. We additionally had vertical and horizontal EOG electrodes placed around the eyes, a respiration belt transducer to measure respiratory rate, a pulse oximeter to measure heart rate, and a second oximeter to measure oxygen saturation levels in the blood (SpO_2), all collected synchronized with the EEG data. 100% Oxygen or room air was delivered through an unsealed gas delivery mask, allowing 2 minutes before the start of each 15-minute block to allow the oxygen to be taken up by the body. Pulse oxygenation level was measured at the start and end of this 2-minute period.

Our data was processed using EEGLAB toolbox functions (Delorne & Makeig, 2004) with custom scripts written in MATLAB. We followed standard protocols in our lab for the preprocessing of EEG data. Raw traces were segmented into 180 second epochs locked to the onset of each trial corresponding to the following conditions: normoxia eyes-opened, normoxia eyes-closed, hyperoxia eyes-opened, and hyperoxia eyes-closed, resulting in four 3-minute epochs within each condition, and 16 trials total per participant. An additional 9s was taken before and after each trial to avoid edge artifacts. Eye movements were corrected with a regression-based procedure (Gratton, Coles, & Donchin, 1983), which is standard procedure for correcting eye movements. Eye movement data were examined visually post-correction to

determine that the regression-based measure was equally effective for both eyes-opened and eyes-closed conditions. No other form of artifact correction was used.

Time frequency Analysis

We transformed our 3-minute segments into time-frequency space by convolving the data with a Morlet wavelet 6 cycles in length and for 100 logarithmically sampled frequencies from 1 – 50Hz. These raw power values were log-transformed and averaged within each segment, resulting in four trial averages within each condition. These trial averages were then themselves averaged, resulting in a measure of the average raw power at each frequency, for each of the 4 conditions, for each electrode, and for each participant.

In addition to the analysis of the oscillatory power, we used the BOSC (Better OSCillation detection) method, introduced by Caplan et al. (2001) and recently revised by Whitten, Hughes, Dickson and Caplan (2011). The BOSC method is designed to detect consistent oscillatory activity in EEG signals by modelling the functional form of the non-rhythmic background and revealing segments of the recording that deviate significantly and consistently from the spectral characteristics of the background. The procedure applies the raw wavelet power values to two thresholding procedures to ensure the presence of oscillatory activity, using one threshold for amplitude and one for duration. First, the average wavelet power across the entire experimental session is used to estimate the spectral background. This background, observed as a 1/f relationship between frequency and power, is a natural property of autocorrelated time-series signals such as EEG (Schlesinger & West, 1988) and is assumed for our purposes to take the form of coloured noise, i.e., described by the equation $\text{Power}(f) = Af^{-\alpha}$. We fit the parameters of this function by log-transforming our session-averaged wavelet spectrum and computing a linear regression of these values on frequency in log-log space,

resulting in an intercept, corresponding to log(A), and a slope, corresponding to -α. It is possible to consider this fitted value (Power(*f*)) as the mean of the theoretical $\chi^2_{(2)}$ distribution, allowing us to assign a probability to the observed power values at a given frequency. As has been done in other applications of the BOSC method (Whitten, Hughes, Dickson & Caplan, 2011; Hughes, Whitten, Caplan, & Dickson, 2012) the power threshold, P_T, is chosen such that only power values exceeding the 95th percentile of this distribution are large enough to be considered oscillatory. The duration threshold, D_T requires that for activity at any given frequency to be considered a "detected oscillation", the wavelet power must exceed D_T for at least 3 cycles of that frequency. The end result is a binary value of "detected" or "undetected" for every point in time-frequency space. The number of detected time-points is then divided by the total time in the segment to obtain the "Pepisode", the proportion of time oscillations of a given frequency were detected in a given period. In our analyses this proportion was multiplied by 100 and presented as a percentage for ease of understanding. Lastly, we averaged the Pepisode from the four trials within each condition, resulting in one value of the mean Pepisode for each frequency, condition, electrode, and participant, mirroring our power analyses. The benefit of this procedure is that it allows our data to explicitly show the relative amount of truly oscillatory activity that was present in the data in a given period of time, as opposed to the average power which can be misleading since it can be influenced by non-oscillatory activity, and since results can be determined by short bursts of high amplitude oscillatory activity that do not accurately reflect the entire period being measured.

For both the raw power and the Pepisode we averaged the values within each condition across all participants and across all electrodes, resulting in a grand-average spectrum that clearly showed that the eyes-closed and eyes-opened trials were dissimilarly affected by the oxygen

manipulation. Examining eyes-closed and eyes-opened trials separately, we performed a cluster-based statistical analysis of the effect of hyperoxia across frequencies and electrodes. At each frequency and each electrode, a two-tailed uncorrected paired t-test was performed with an alpha set to 0.05. In order to minimize false alarms, we considered there to be a significant difference at that frequency if at last three clustered electrodes showed a significant difference. We defined an electrode cluster as a spatial array of at least three mutually adjacent electrodes showing significant differences in a common direction. Based on this criterion, bands of frequencies were identified which consistently showed an effect of hyperoxia across similar clusters of electrodes, and the t-statistics for each electrode were averaged across the frequencies within the band and plotted as a scalp topography to show to spatial localization of the statistical effect. Significant electrodes were marked on the topography with a green asterisk. For each topography, we selected the electrode with the largest t-statistic and plotted a bar graph to show the actual power or Pepisode values in the hyperoxia and normoxia conditions. A final uncorrected two-tailed paired t-statistic for the mean power or Pepisode difference within this frequency range and at this single electrode was calculated and reported. As they are derived from the most significant electrode from a group of significant electrodes, this test-statistic and the accompanying bar-graph should be considered an inflated measure of the true effect size. The difference spectra, hyperoxia minus normoxia, was also computed and plotted, to present at a single electrode the magnitude and variability of the difference hyperoxia produces across the spectrum. The electrode chosen was using the electrode with the largest t-statistic, but in some cases was chosen to best reflect the most theoretically interesting results for that condition.

In addition to examining the effect of hyperoxia on the eyes-opened and eyes-closed trials separately, we were also interested in the effect of hyperoxia on the transition between one

eye-condition to the next. In order to examine this, we computed the average wavelet power within each of the four oxygen conditions (2 trials of hyperoxia, 2 trials of normoxia), instead of across the whole session. This average power was used as a baseline from which to subtract the eyes-opened and eyes-closed power from that condition, and was also used to estimate separate P_T thresholds within each oxygen condition and recompute the Pepisode measure for each eye-condition. This analysis allowed the relative power and Pepisode to be compared within oxygen blocks without being affected by the variability between the blocks. As was done previously, we computed grand-average spectra across participants and electrodes, and then proceeded to look for frequencies and electrodes which showed significant interactions between the oxygen and eye conditions. At every electrode and frequency, a two-way repeated-measures ANOVA was performed, with an alpha set to 0.05, and using the uncorrected F-statistic for the eye by oxygen interaction as our indication of significance across electrodes. As before, only frequencies with clusters of at least 3 electrodes were considered to be significant. Bands of frequencies were identified showing a significant interaction between the magnitude of the eyes-opened/eyes-closed difference and the inhaled oxygen, and the F-statistics within these bands were averaged at each electrode and plotted on a topography to show the spatial localization of the interaction. Significant electrodes were marked on the topography with a green asterisk, and bar graphs were plotted to show the actual power or Pepisode values in all four conditions, at the electrode with the largest F-statistic. The difference spectra, eyes-closed minus eyes-opened, was plotted for both the hyperoxia and the normoxia conditions, averaged across repeated trials, to show at a single electrode the magnitude and variability of the interaction. The electrode chosen was in all cases the electrode with the largest F-statistic.

We performed two additional analyses with the aim of examining in more detail how the patterns of oscillatory activity were changing between the conditions. The first analysis consisted of a restricted power analysis where time points not classified as “detected” within a given frequency were not considered in the estimation of the oscillation power. Oscillation power is often interpreted as reflecting the amplitude of an oscillation, but since power is estimated on both oscillatory and non-oscillatory activity within a time segment, this is not necessarily true. If the proportion of that segment which is oscillatory increases or decreases, then the estimation of power across the whole segment will also increase or decrease even when the amplitude of the oscillatory portion remains constant. We returned to the frequency bands which showed a significant effect of hyperoxia on the Pepisode within an eye condition, and re-estimated the power in these ranges by averaging across only detected oscillations within a trial, and then averaging this value across the 4 trials per condition as above to obtain one measure of the “detected raw power” within each condition which is not affected by differences in Pepisode between conditions. This measure was calculated only at frequencies and electrodes already shown in the previous analyses so as to present data comparable to that presented in the bar graphs in Figure 2B, C, and G. An uncorrected two-tailed paired t-test was performed on the detected power within this range and the means, standard deviations, and t-statistics for this test are reported.

We lastly examined whether the differences observed due to both factors could be explained by increases and decreases in the overall cycle length, or rather by increased number of oscillatory segments of a consistent length. Cycle length was defined as the duration that the oscillatory segment remained over the power threshold P_T, and excluding durations less than 3 cycles for failing to meet the minimum duration threshold D_T. This analysis was restricted to the

observed interaction between oxygen condition and eye condition in the alpha range presented in Figure 2G. The alpha frequency range (7-14Hz) contains by far the most commonly detected oscillations in our data and is the only range which contains enough separate oscillatory epochs to allow a stable estimation of the oscillatory epoch length. We first identified the number and duration of every detected oscillatory epoch, within the frequency range previously identified as showing an interaction between oxygen condition and eye condition on the detection of alpha oscillations, and at the electrode with the largest test statistic for this interaction. We pooled the oscillatory epoch counts across trials and across participants, due to the fact that the number of oscillatory epochs per subject was still typically less than 30 – not enough to reliably estimate the epoch length distribution within participants. We first plotted histograms of the cycle lengths from the eyes-opened and eyes-closed conditions, overlaid, so as to demonstrate the shift in the alpha cycle length distribution that is caused by closing the eyes. Separate pairs of histograms were plotted for the hyperoxia and normoxia conditions. To compare all four distributions, we estimated the cumulative distribution function (cdf) for these four distributions using the MATLAB ecdf() function, which uses a method derived from survival analysis called the Kaplan-Meier nonparametric method to empirically estimate the cumulative distribution function of a population from observed data. We plotted the cdf for each condition on one axis to observe the impact of hyperoxia on the eyes-opened and eyes-closed cycle length distributions. We tested for significant differences between the hyperoxia and normoxia conditions using the two-sample Kolmogorov-Smirnov (KS) test, which determines if two independent samples come from identical distributions. We used one-tailed tests to correct for the high sensitivity of the KS test. Specifically, we tested if in the eyes-opened condition the hyperoxia distribution would be shifted more towards shorter cycle lengths than the normoxia distribution (left-tailed test), and in

the eyes-closed condition that the hyperoxia distribution would be shifted towards longer cycle lengths (right-tailed test).

Results

Physiological measurements

Exposure to hyperoxia elevated pulse oxygen saturation levels significantly across participants (Figure 2A), demonstrating that the oxygen delivery was effective in our participants. Consistent with the literature, hyperoxia had a slight but non-significant slowing effect on breathing rate in eyes-opened ($t(25) = .968$, *n.s.*) and eye-closed conditions ($t(25) = .701$, *n.s.*; Figure 2B), and significantly lowered heart rate in both the eyes-opened ($t(25) = 4.87$, $p < 0.001$) and eyes-closed ($t(25) = 3.06$, $p < 0.01$) conditions (Figure 2C).

Power Analyses

Across all electrodes, a large decrease in power was observed in the alpha band in the eyes-opened condition compared to the eyes-closed condition (Figure 3A), as would be expected from previous research on alpha oscillations (Heagens et al., 2014). We performed a statistical analysis to isolate clusters of frequencies and electrodes showing reliable power differences (Bew, Field, Droste, & Razis, 1994; Desai, Tailor, & Bhatt, 2015; Kety & Schmidt, 1948; Pack, Cola, Goldszmidt, Ogilvie, & Gottschalk, 1992; Tsanov, Chah, Reilly, & O'Mara, 2014) between the hyperoxia and normoxia conditions. In the eyes-opened condition, hyperoxia was associated with decreases in power in the alpha (7.95 – 9.66 Hz) and beta (17.38 – 21.98 Hz) frequency ranges, as shown in the electrode by frequency heatmap plot in Figure 3B. Test statistics whose probability is below the 0.05 alpha level are emphasized in darker colours. The decrease in alpha power was focused around fronto-central electrode sites (electrode F3: $M_{hyp} = 5.8215$, $M_{norm} = 5.8542$; $M_{diff} = 0.0328$, $SD_{diff} = 0.0748$; $t(25) = -2.23$, $p = 0.035$). The decrease

in beta power was focused around left- frontal sites. (electrode F3: M_{hyp} = 5.1584, M_{norm} = 5.1900; M_{diff} = 0.0316, SD_{diff} = 0.0684; $t(25) = -2.36$, $p = 0.027$). The difference spectra, hyperoxia – normoxia, shows the impact of hyperoxia on the oscillations at electrode F3. The shaded lines represent the 95% CI for the difference. Frequencies in the alpha and beta frequency ranges which reached significance at this level are indicated in red on the x-axis (listed above; Figure 3D). In the eyes-closed condition, hyperoxia was not associated with any reliable changes in power (Figure 3C).

Figure 3E shows the power in the eyes-opened and eyes-closed conditions after the average power within the entire oxygen block was subtracted out. It can be observed that within the hyperoxia blocks, the difference in power between eyes-closed and eyes-opened was more pronounced. We isolated the clusters of electrodes and frequencies which reliably showed this interaction between the magnitude of the eyes-opened and eyes-closed difference and the oxygen condition, revealing one band in the alpha range from 7.64 – 8.94 Hz, as shown in the electrode by frequency heatmap in Figure 3F. This interaction was present in a large group of frontal electrode sites. (electrode F3: $M_{Hypdiff}$ = 0.2496, $M_{Normdiff}$ = 0.2032; $SD_{hypdiff}$ = 0.2305, $SD_{normdiff}$ = 0.1983; $F(1,25) = 6.1031$, $p = 0.021$). The two difference spectras, eyes-closed – eyes-opened, were plotted for both the hyperoxia and normoxia conditions at electrode F3 (Figure 3G). Error bars represent the within-subject SEM, removing the variance associated with differences between individuals (Loftus & Mason, 1994). The frequencies in the alpha range for which the oxygen by eye interaction reaches significance at the 0.05 alpha level are indicated in red on the x-axis (indicated above).

BOSC and Pepisode Analyses

Because spectral power reflects a smeared average across frequency bands and is not necessarily a robust method for detecting oscillations per se (Caplan et al., 2001), we used the Better OSCillation (BOSC) detection method which has been validated for state-dependent alternations in alpha detections (Whitten et al, 2011). Across all electrodes, a large (~20%) decrease in the number of detected alpha oscillations was observed in the eyes-opened condition compared to the eyes-closed condition (Figure 4A), similar to the power results. We performed the same statistical analysis to isolate clusters of frequencies and electrodes showing reliable differences in Pepisode between the hyperoxia and normoxia conditions. In the eyes-opened condition, hyperoxia was associated with decreases in Pepisode in the alpha (6.80 – 10.06 Hz), beta (14.30 – 19.55 Hz), and gamma (21.14 – 35.16 Hz) frequency ranges, as shown in the electrode by frequency heatmap plot in Figure 4B. Test statistics which are below the 0.05 alpha level are emphasized in darker colours. The decrease in alpha power was again focused around frontal electrode sites, and was significant in a larger number of sites than in the power analysis. (electrode F3: M_{hyp} = 8.00, M_{norm} = 9.62; M_{diff} = 1.61, SD_{diff} = 2.78; $t(25) = -2.74$, p = 0.0067. The decrease in beta power was focused around left-frontal sites. (electrode F3: M_{hyp} = 2.18, M_{norm} = 2.74; M_{diff} = 0.56, SD_{diff} = 0.91; $t(25) = -3.13$, p = 0.0044). The decrease in gamma power was focused around a range of sites over the left-frontal, central, and posterior regions. (electrode Pz (M_{hyp} = 0.70, M_{norm} = 1.00; M_{diff} = 0.31, SD_{diff} = 0.60; $t(25) = -2.60$, p = 0.015). The difference spectra, hyperoxia – normoxia, shows the impact of hyperoxia on the oscillations at electrode F3. Error bars represent the 95% CI for the difference. Frequencies in the alpha, beta, and gamma frequency ranges which reached significance at this level are indicated in red on the x-axis (listed above; Figure 4D).

In the eyes-closed condition, hyperoxia was associated with increases in Pepisode in the delta (1.73 – 2.10 Hz), and theta (4.25 – 4.60 Hz) frequency ranges, and with a decrease in the beta (15.46 – 19.55 Hz) frequency ranges, in contrast to the lack of differences found in the power analysis (Figure 4C). Test statistics whose probability were below the 0.05 alpha level are emphasized in darker colours. The increase in detected delta oscillations was focused around right-posterior electrode sites. (electrode PO4: M_{hyp} = 0.76, M_{norm} = 0.36; M_{diff} = 0.40, SD_{diff} = 0.56; $t(25) = 3.63$, p = 0.0013). The increase in detected theta oscillations was focused around a small cluster of right-frontal sites. (electrode F8: M_{hyp} = 0.75, M_{norm} = 0.46; M_{diff} = 0.29, SD_{diff} = 0.46; $t(25) = 3.22$, p = 0.0036). The decrease in detected beta oscillations was focused around frontal and central electrode sites. (electrode C4: M_{hyp} = 3.92, M_{norm} = 4.44; M_{diff} = 0.52, SD_{diff} = 1.05; $t(25) = -2.52$, p = 0.018). The difference spectra subtracting hyperoxia from normoxia, shows the impact of hyperoxia on the oscillations at electrode F3. Error bars represent the 95% CI for the difference. Frequencies in the alpha, beta, and gamma frequency ranges which reached significance at this level are indicated in red on the x-axis (listed above; Figure 4E).

Figure 4F shows the Pepisode in the eyes-opened and eyes-closed conditions when the power threshold was estimated within an oxygen condition rather than across the whole experimental session. In this case, the oscillation detection procedure does not seem to depend as strongly as the power analysis on the choice of baseline. We isolated the clusters of electrodes and frequencies which reliably showed an interaction between the magnitude of the eyes-opened and eyes-closed difference and the oxygen condition, revealing one band in the alpha range (6.80 – 9.30 Hz), and one band in the gamma range (23.78 – 42.76 Hz), as shown Figure 4G. The interaction in the alpha range was present across most of the scalp and especially in frontal and central electrode sites. (electrode F3: $M_{Hypdiff}$ = 13.43, $M_{Normdiff}$ = 10.19; $SD_{hypdiff}$ = 12.83,

$SD_{normdiff}$ = 11.33; $F(1,25) = 10.58$, $p = 3.0\times10^{-5}$). The interaction in the gamma range was present across a large group of electrodes centered on left-central electrode sites. (electrode FC1: $M_{Hypdiff} = 0.026$, $M_{Normdiff} = -0.42$; $SD_{hypdiff} = 1.09$, $SD_{normdiff} = 1.47$; $F(1,25) = 5.50$, $p = 0.036$). The two difference spectras, eyes-closed – eyes-opened, were plotted for both the hyperoxia and normoxia conditions at electrode F3 (Figure 4H). Error bars represent the within-subject SEM. Frequencies in the alpha and gamma range for which the oxygen by eye interaction reaches significance at the 0.05 alpha level are indicated in red on the x-axis.

Analyses of detected alpha epochs

For periods of detected oscillatory activity in our frequency bands of interest, we computed the mean raw power to determine if there were any differences in the amplitude of the signals as an effect of hyperoxia (Figure 5A). For five out of our six bands, although differences had been observed in the number of detected oscillations, no change in detected power was observed between hyperoxia and normoxia conditions. For the remaining band, however (21.14 – 35.16 Hz) in the eyes-opened condition, the decrease in detected oscillations was accompanied by a decrease in detected power.

We also characterized the actual number of cycles in each detected alpha epoch to determine if hyperoxia had any effect on epoch lengths. Figure 5B shows the histograms of the pooled cycle lengths from the alpha range frequencies shown in Figure 4G showing a significant interaction between eye and oxygen condition. Only detected segments from electrode F3 were included. For both hyperoxia and normoxia conditions, opening the eyes leads to a larger proportion of short (3-6 cycle) oscillatory periods, while closing the eyes increases the proportion of long (7-25 cycle) oscillatory periods. Figure 5C shows the empirical cumulative distribution function (cdf) derived from the pooled cycle lengths. This figure similarly shows that

closing the eyes leads to a cdf which is more shifted to the longer cycles lengths. This effect is larger for hyperoxia than normoxia. The KS test for the hypothesis that the hyperoxia eyes-opened condition was significantly “smaller” than the distribution for the normoxia condition resulted in a p-value of 1.54×10^{-7}, whereas the test for the hypothesis that the hyperoxia eyes-closed condition was significantly “larger” than the distribution for the hyperoxia eyes-opened condition resulted in a p-value of 5.84×10^{-8}.

Discussion

Oxygen is used in many clinical settings and its popularity as a recreational activity is rising. Our aim was to evaluate possible attentional benefits of short term oxygen administration through an eyes-closed and eyes-opened resting-state EEG task. Under short-term hyperoxia, we observed changes in blood O_2 saturation as well as in heart and ,to a lesser extent, breathing rates. In addition, in the brain, there were state-dependent decreases across several high EEG frequency bands, including low alpha, high alpha, beta, and gamma, as well as increases in low frequency bands including delta and theta. Finally, our results also demonstrate interactions between the oxygen and eye-conditions, which suggests that the effects of oxygen on cortical activity may be state-specific.

Our experiment measured SpO_2, breathing rate, and heart rate. Breathing rate is often ignored in studies that examine cognitive tasks under hyperoxia. Monitoring for breathing rate is useful to elucidate whether changes in spontaneous brain activity are due to oxygen manipulations per se or instead influenced by fluctuating CO_2 levels, which can be affected by breathing rate. Since there were increases in SpO_2, but little change in breathing rate between the oxygen conditions, the changes in brain activity observed are most likely due to the oxygen

manipulation rather than change in central CO_2. Our finding of decreased heart rate with oxygen administration is consistent with the literature and suggests that oxygen influences the parasympathetic nervous system (Shibata, Iwasaki, Ogawa, Kato, & Ogawa, 2005; Waring et al., 2013). The reduction in parasympathetic activity is potentially mediated by a reduced input from peripheral chemoreceptors to the medulla oblongata (Lahiri, Mokashi, Mulligan, & Nishino, 1981). Since we made no measurements of cerebral blood flow changes, we cannot eliminate this as a possible factor in the changes we observed in brain activity, although a recent MRI experiment found no evidence that short-term hyperoxia causes such changes (Croal et al., 2015) and a variety of sleep studies using PET have shown evidence of uncoupling of cerebral blood flow and EEG measures of brain state (Bangash et al. 2008; Braun et al. 1997; Hajak et al. 1994; Hofle et al. 1997). .

Based on the known associations of alpha with visual and visuo-spatial attention (Thut et al., 2006; Klimesch, Sauseng, & Hansylmayr, 2007; Jensen & Mazaheri, 2010; Mathewson et al., 2011; Kizuk & Mathewson, 2017), we would hypothesize that fewer alpha detections and lower power during eyes-opened conditions with hyperoxia would produce a beneficial effect for these processes. Similar to the functional role of alpha in visual and other sensory modalities, a suppression of beta activity is often associated with the role of sensory gating in the somatosensory system and anticipatory selective attention (Neuper, Wörtz, & Pfurtscheller, 2006; Pomper, 2015). Our finding of a robust difference in beta activity between normoxia and hyperoxia when the eyes were open but less so when the eyes were closed could suggest that the impact of oxygen affects beta during alert, attentional states, and has little effect with a disengaged state with the eyes-closed. This decrease in alpha and beta activity under hyperoxia during alert states lends credence to reports of noticeable attentional changes when using

recreational oxygen, given the growing literature on beta activity and cognitive functioning. Engel and Fries (2010) propose that a functional role of beta activity is the maintenance of the current cognitive state of the participant. Under this framework, the decreases in the observed beta activity under hyperoxia could reflect increased cognitive control and attentional flexibility, which users of recreational oxygen interpret as improving their attention. Perhaps paradoxically, although supported by previous studies which likely recorded under eyes-closed conditions (Seo et al., 2007; Wu et al., 2014), hyperoxia appeared to enhance delta and theta activities while decreasing gamma, a result that would likely indicate promotion of the initial stages (i.e., N1) of sleep (Borbély & Tobler, 2011; Greene & Frank, 2010).

The eyes-closed and eyes-opened resting-state EEG task allowed us to compare within and across different attentional states. As expected, opening and closing the eyes produced clear differences in both the power and the Pepisode (frequency of detection) of alpha oscillations. When the eyes were open, the detections of alpha, beta, and gamma bandwidth oscillations decreased when hyperoxia was administered, whereas when the eyes were closed, the detections of delta and theta bandwidth oscillations increased with hyperoxia, along with a decrease in gamma detections. Hyperoxia was associated with a more notable decrease in alpha power and both alpha and gamma detections when the eyes were opened compared to when they were closed. In addition, we observed that hyperoxia contributes to the magnitude of the transition between eyes-opened (engaged) and eyes-closed (disengaged) oscillatory states. There was a significant interaction between oxygen and eye effects such there was a significantly larger transition during hyperoxia in both the power and detection of alpha oscillations across the eyes closed/opened condition than during normoxia. Indeed, when we computed the average number of contingous cycles of alpha using BOSC, we observed that not only did closing the eyes

produce longer bursts of alpha activity, but that hyperoxia was associated with a more exaggerated transition across the behavioural condition.

While different oscillatory states are associated with specific cognitive functions, our task did not specifically test a cognitive function, rather it simply demonstrated that there are oscillatory changes that occur under short-term hyperoxia in resting state conditions. Sheng et al. (2017) recently demonstrated that hyperoxia was associated with reductions in alpha and beta power during both a sustained attention task and a visual ERP task. Our results, similarly showed that hyperoxia was associated with decreases in alpha and beta power as well as proportion of detections during a resting state task when the eyes were opened but extended this to the comparison of eyes-closed condition in which this decrease was lessened.

That said, there are other factors which may have influenced our findings. In particular, the fronto-temporal localization of the beta and gamma decrease under hyperoxia suggest that decreased scalp and eye muscular activity could be contributing in whole or in part to our results. This lays the foundation for future research to explore the potential of transient administration of oxygen on attentional tasks, to see if these changes in oscillatory dynamics correlate with behavioural performance. Such studies should be careful to measure muscular contributions to oscillation measures using EMG. Another limitation of our study was that our SpO_2 measurement was not continuously assessed during the trials, but rather at the beginning and ends of each block. Thus, we were only able to examine differences in SpO_2 across different blocks. Lastly, we were unable to assess whether participant entered into light stages of sleep during the eyes-closed condition, which may have contributed to the enhancements seen in delta and theta bands.

Conclusion

Here we manipulated oxygen administration in eyes-opened and eyes-closed resting states while monitoring for respiration and other physiological responses like heart rate and SpO_2. Modest EEG changes were seen in both resting state conditions during hyperoxia that may be associated with increased alertness during eyes-opened conditions and paradoxically perhaps, the initiation of sleep states during eyes-closed conditions. In addition, we found that oxygen enhanced the changes typically seen when comparing eyes-opened to eyes-closed conditions, especially in the alpha band. Our results suggest that the administration of oxygen could potentially have the attentional benefits specified by recreational oxygen providers during eyes-opened wakefulness and may also differentially promote relaxation and sleep during eyes-closed conditions, although future studies are needed to fully evaluate the effects of oxygen on attentional processing, behavioural performance, and behavioural state.

Acknowledgements

This work was supported by Natural Science and Engineering Research Council of Canada (NSERC) discovery grants 2016-06576 and 04792 to Drs. Clayton T Dickson and Kyle E. Mathewson, respectively. This work was also supported by a Branch Out Neurological Foundation summer scholarship to Wesley Vuong. Sayeed Kizuk was also partially supported by the Neuroscience and Mental Health Institute of the University of Alberta.The authors would also like to extend a warm appreciation to members of the Attention Perception and Performance Lab and the Brain Rhythms Lab for helping with experiments.

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Figure Captions

Figure 1. Experimental procedure. Participants completed four 3-minute trials, two eyes-opened and two eyes-closed, within each gas block. Participants completed four gas blocks in total, two hyperoxia and two normoxia conditions, interleaved. Block order was counterbalanced across subjects.

Figure 2: Physiological measurements. A) SpO2 measurements for the baseline period (red) and for the average of the two measurements at the beginning and end of each gas block (blue). B) Breathing rate differences between hyperoxia and normoxia conditions. No significant within-subjects difference was observed. Dark line is grand average, error bars are within-subject SEM. C) Heart rate differences between hyperoxia and normoxia conditions. Heart rate was significantly lower in the hyperoxia condition for both eyes-opened ($p < 0.001$) and eyes-closed ($p < 0.01$) conditions.

Figure 3: Analysis of power changes across electrodes, frequencies, state, and oxygen conditions. A) The raw power spectra of each condition averaged across all electrodes. Shaded lines represent the within-subject SEM. B) The t-statistic for the difference due to hyperoxia on eyes-opened power is plotted at each electrode and frequency. Comparisons which were statistically significant at the 0.05 α level are emphasized. Topographical distributions of the statistical effects, as well as the magnitude of power in each condition is plotted for each band. Error bars represent the within-subject SEM. C) The t-statistic for the difference due to hyperoxia on eyes-closed power is plotted at every electrode and frequency, showing no clusters of significant differences. D) The difference spectrum of hyperoxia – normoxia power for the eyes-opened condition, from electrode F3. The shaded line plots the 95% CI for the difference. E) Power spectra for each condition after

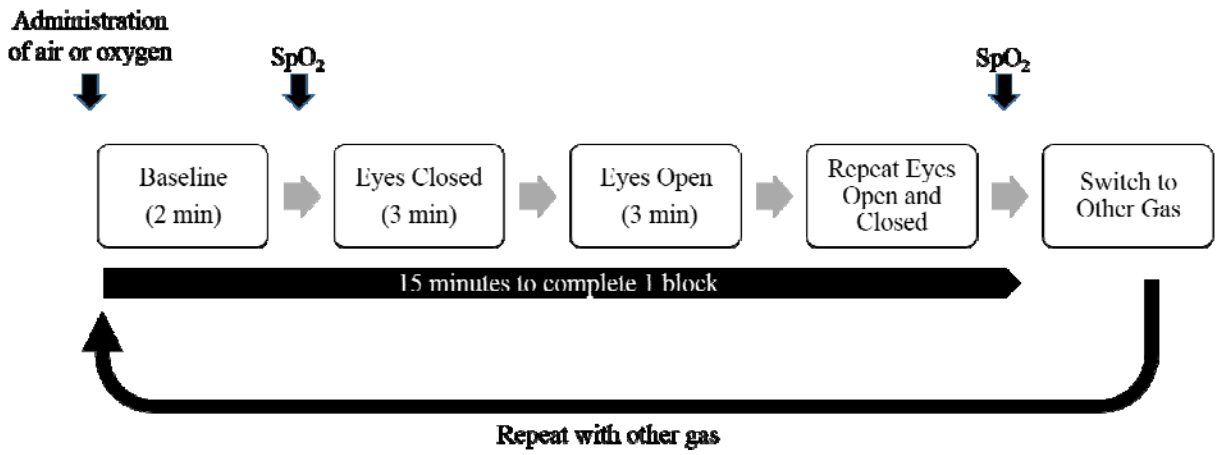
subtracting the overall average power across conditions, accentuating the differences due to gas. F) The F statistic for the interaction between gas condition and eye condition is plotted at every electrode and frequency. Comparisons which were statistically significant at the 0.05 α level are emphasized. The topographical distribution of the statistical effect in this band as well as the magnitude of the raw power in each condition are plotted for each condition. Error bars represent the within-subject SEM. G) The difference spectra for eyes-closed – eyes-opened power across the hyperoxia and normoxia conditions, for electrode F3. Error bars represent the within-subject SEM. As shown, a larger alpha amplitude difference exists in the hyperoxic state between eyes-closed and eyes-opened conditions.

Figure 4: Analysis of changes in detected oscillations across electrodes, frequencies, state, and oxygen conditions. A) The Pepisode spectra across all electrodes, thresholded with the average power spectrum across the whole experiment. Error bars represent the within-subject SEM. B) The t-statistic for the difference due to hyperoxia on eyes-opened Pepisode is plotted at each electrode and frequency. Comparisons which were statistically significant at the 0.05 α level are emphasized. The topographical distributions of the statistical effect, as well as the percentage of time oscillations were detected in each condition is plotted for each band. Error bars represent the within-subject SEM. C) The same analysis as for B) is presented for the eyes-closed condition. D) The difference spectrum of hyperoxia – normoxia Pepisode during the eyes-opened condition, from electrode F3. The shaded error plot represents the 95% CI for the difference. E) The difference spectrum of eyes-closed Pepisode, hyperoxia – normoxia, plotted from electrode PO4 to show the observed increase in the delta band. The error bar represents the 95% CI for the difference. F) The Pepisode spectra thresholded with the averaged power within

each gas condition, rather than across all gas conditions showing that the choice of background spectra did not influence the detection of oscillations of different frequencies. G) The F statistic for the interaction between gas condition and eye condition is plotted at every electrode and frequency. Comparisons which were statistically significant at the 0.05 α level are emphasized. The topographical distribution of the statistical effect in this band as well as the percentage of time oscillations were detected are plotted for each condition. Error bars represent the within-subject SEM. H) The difference spectra for eyes-closed – eyes-opened Pepisode across the hyperoxia and normoxia conditions, for electrode F3. This shows a larger difference in the hyperoxia condition. Error bars represent the within-subject SEM.

Figure 5: Analysis of detected alpha epoch lengths. A) Bar graphs showing the raw power values within detected segments only (detected power) for the six frequency ranges which showed main effects of gas on detected oscillations. Only the beta frequency range of 21.14 – 35.16 Hz in the eyes-opened condition shows a decrease in power within detected segments. Error bars are within-subject SEM. B) Cycle length distributions for detected cycles from the range of 6.8 : 9.3 Hz. Closing the eyes results in longer cycle lengths, for both normoxia and hyperoxia conditions. C) Empirical cumulative distribution function estimated from the pooled cycle length distributions across subjects. Hyperoxia cdfs were significantly more extreme than normoxia cdfs.

Figure 1


Administration of air or oxygen
SpO2
SpO2
Baseline (2 min)
Eyes Closed (3 min)
Eyes Open (3 min)
Repeat Eyes Open and Closed
Switch to Other Gas
15 minutes to complete 1 block
Repeat with other gas

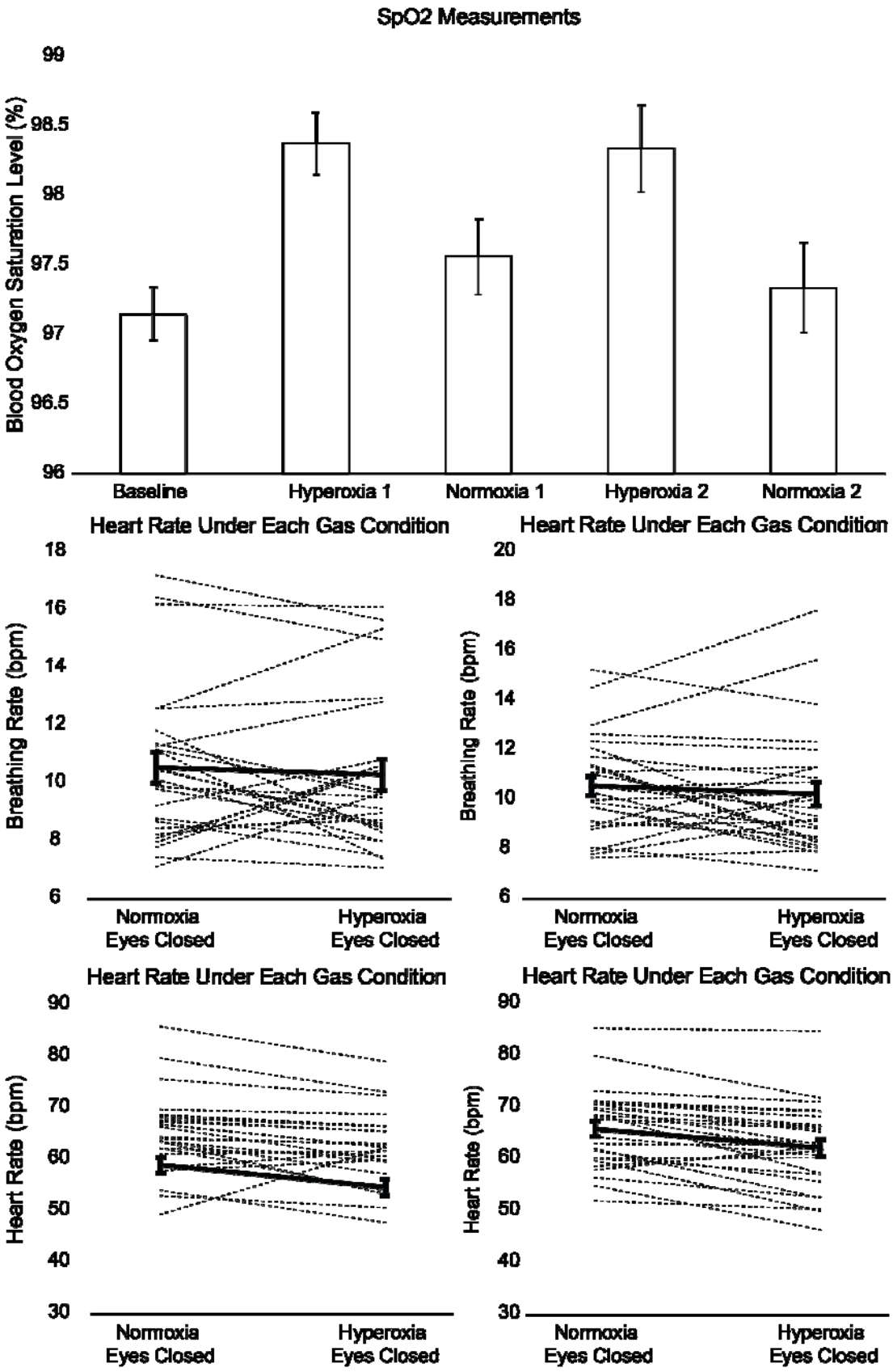

SpO2 Measurements
Blood Oxygen Saturation Level (%)
99
98.5
98
97.5
97
96.5
96
Baseline
Hyperoxia 1
Normoxia 1
Hyperoxia 2
Normoxia 2
Heart Rate Under Each Gas Condition
Breathing Rate (bpm)
18
16
14
12
10
8
6
Normoxia Eyes Closed
Hyperoxia Eyes Closed
Heart Rate Under Each Gas Condition
Breathing Rate (bpm)
20
18
16
14
12
10
8
6
Normoxia Eyes Closed
Hyperoxia Eyes Closed
Heart Rate Under Each Gas Condition
Heart Rate (bpm)
90
80
70
60
50
40
30
Normoxia Eyes Closed
Hyperoxia Eyes Closed
Heart Rate Under Each Gas Condition
Heart Rate (bpm)
90
80
70
60
50
40
30
Normoxia Eyes Closed
Hyperoxia Eyes Closed

Figure 2

Figure 3

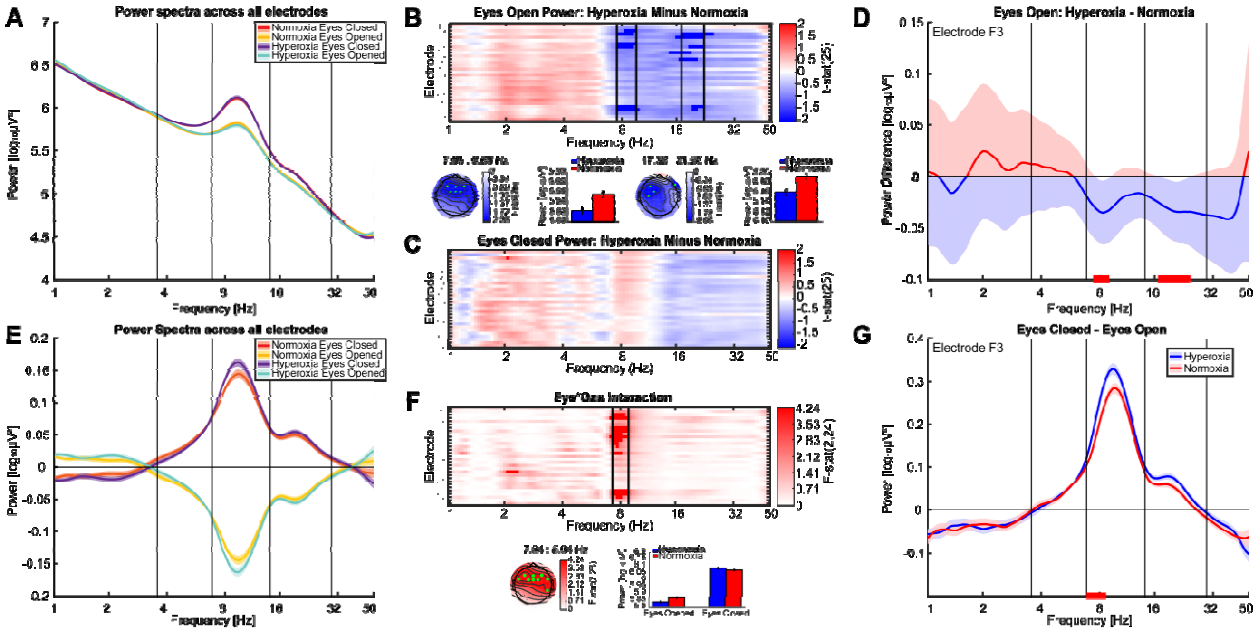

A
Power spectra across all electrodes
Normoxia Eyes Closed
Normoxia Eyes Opened
Hyperoxia Eyes Closed
Hyperoxia Eyes Opened
Frequency [Hz]
B
Eyes Open Power: Hyperoxia Minus Normoxia
Electrode
Frequency (Hz)
C
Eyes Closed Power: Hyperoxia Minus Normoxia
D
Eyes Open: Hyperoxia - Normoxia
Electrode F3
Power Difference [log10 µV²]
E
Power Spectra across all electrodes
F
Eye*Oxa Interaction
G
Eyes Closed - Eyes Open
Hyperoxia
Normoxia

Figure 4

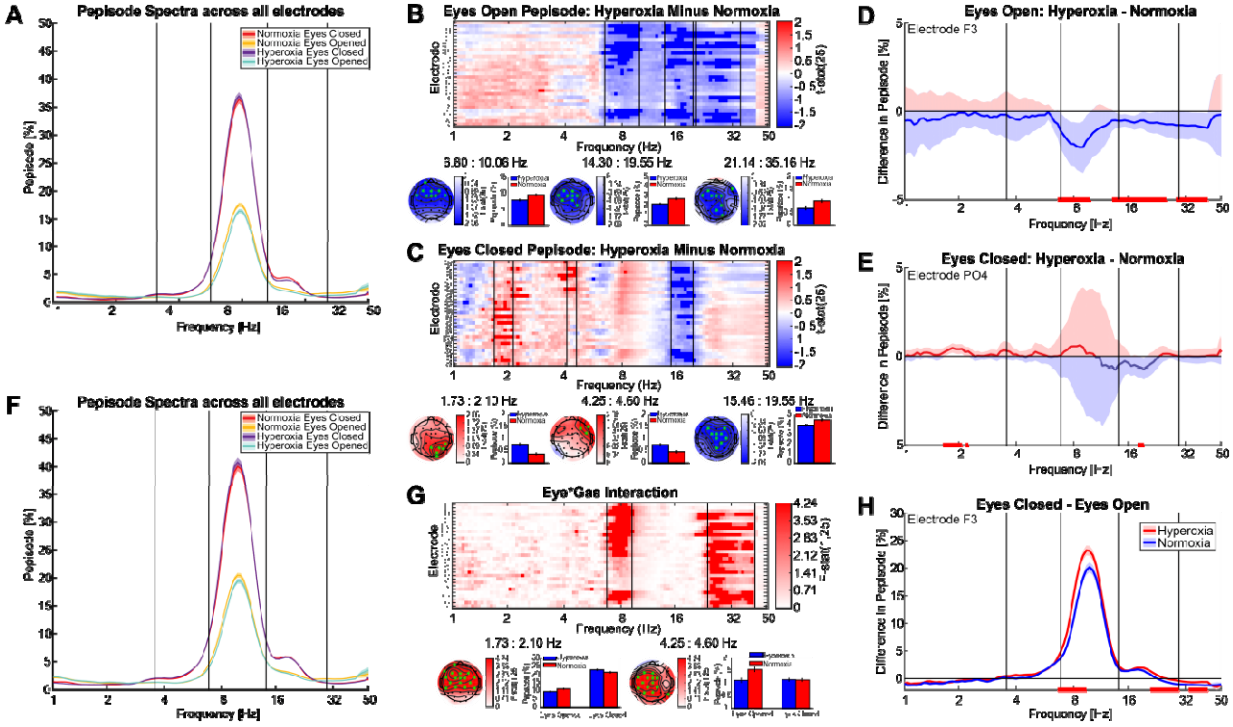
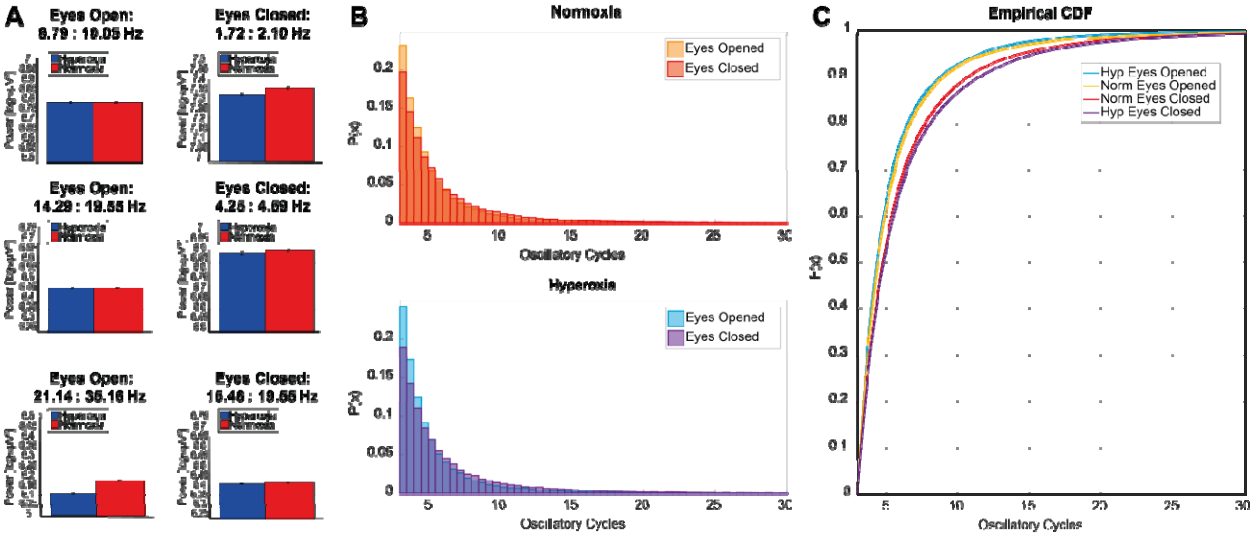

A
Epsisode Spectra across all electrodes
Normoxia Eyes Closed
Normoxia Eyes Opened
Hyperoxia Eyes Closed
Hyperoxia Eyes Opened
Epsisode [%]
Frequency [Hz]
B
Eyes Open Epsisode: Hyperoxia Minus Normoxia
Electrode
Frequency (Hz)
3.80 : 10.06 Hz
14.30 : 19.55 Hz
21.14 : 35.16 Hz
C
Eyes Closed Epsisode: Hyperoxia Minus Normoxia
Electrode
Frequency (Hz)
1.73 : 2.10 Hz
4.25 : 4.60 Hz
15.46 : 19.55 Hz
D
Eyes Open: Hyperoxia - Normoxia
Electrode F3
Difference in Epsisode [%]
Frequency [Hz]
E
Eyes Closed: Hyperoxia - Normoxia
Electrode PO4
Difference in Epsisode [%]
Frequency [Hz]
F
Epsisode Spectra across all electrodes
Normoxia Eyes Closed
Normoxia Eyes Opened
Hyperoxia Eyes Closed
Hyperoxia Eyes Opened
Epsisode [%]
Frequency [Hz]
G
Eye*Gas Interaction
Electrode
Frequency (Hz)
1.73 : 2.10 Hz
4.25 : 4.60 Hz
H
Eyes Closed - Eyes Open
Electrode F3
Hyperoxia
Normoxia
Difference in Epsisode [%]
Frequency [Hz]

Figure 5


A
Eyes Open:
8.79 : 19.05 Hz
Eyes Closed:
1.72 : 2.10 Hz
Eyes Open:
14.29 : 19.55 Hz
Eyes Closed:
4.25 : 4.69 Hz
Eyes Open:
21.14 : 35.16 Hz
Eyes Closed:
16.46 : 19.55 Hz
B
Normoxia
Eyes Opened
Eyes Closed
P(x)
Oscillatory Cycles
Hyperoxia
Eyes Opened
Eyes Closed
P(x)
Oscillatory Cycles
C
Empirical CDF
Hyp Eyes Opened
Norm Eyes Opened
Norm Eyes Closed
Hyp Eyes Closed
F(x)
Oscillatory Cycles