The effect of isocapnic hyperoxia on neurophysiology as measured with MRI and MEG.

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ABSTRACT

The physiological effect of hyperoxia has been poorly characterised, with studies reporting conflicting results on the role of hyperoxia as a vasoconstrictor. It is not clear whether hyperoxia is the primary contributor to vasoconstriction or whether induced changes in CO_2 that commonly accompany hyperoxia are a factor. As calibrated BOLD fMRI based on hyperoxia becomes more widely used, it is essential to understand the effects of oxygen on resting cerebral physiology. This study used a RespirAct^{TM} system to deliver a repeatable isocapnic hyperoxia stimulus to investigate the independent effect of O_2 on cerebral physiology, removing any potential confounds related to altered CO_2. T_1-independent Phase Contrast MRI was used to demonstrate that isocapnic hyperoxia has no significant effect on carotid blood flow (normoxia 201 ± 11 ml/min, -0.3 ± 0.8 % change during hyperoxia, p = 0.8), whilst Look Locker ASL was used to demonstrate that there is no significant change in arterial cerebral blood volume (normoxia 1.3 ± 0.4 %, -0.5 ± 5 % change during hyperoxia). These are in contrast to significant changes in blood flow observed for hypercapnia (6.8 ± 1.5 %/mmHg CO_2). In addition, magnetoencephalography provided a method to monitor the effect of isocapnic hyperoxia on neuronal oscillatory power. In response to hyperoxia, a significant focal decrease in oscillatory power was observed across the alpha, beta and low gamma bands in the occipital lobe, compared to a more global significant decrease on hypercapnia. This work suggests that isocapnic hyperoxia provides a more reliable stimulus than hypercapnia for calibrated BOLD, and that previous reports of vasoconstriction during hyperoxia probably reflect the effects of hyperoxia-induced changes in CO_2. However, hyperoxia does induce changes in oscillatory power consistent with an increase in vigilance, but these changes are smaller than those observed under hypercapnia. The effect of this change in neural activity on calibrated BOLD using hyperoxia or combined hyperoxia and hypercapnia needs further investigation.

Key words: Hyperoxia, Magnetoencephalography, BOLD, fMRI, Cerebral Blood Flow, Cerebral Blood Volume, Neural oscillations.

INTRODUCTION

Hyperoxia (raising the inspired fraction of oxygen (FiO_2) above normal physiological levels (0.21)), is increasingly being used to provide exogenous contrast in functional magnetic resonance imaging (fMRI), primarily to provide a method of calibrating the blood oxygenation level dependent (BOLD) effect, but also to study venous blood oxygenation and venous blood volume (Blockley et al., 2012, Kwong et al., 1995, Rostrup et al., 1995, Bulte et al., 2007a, Bulte et al., 2007b, Chiarelli et al., 2007a, Chiarelli et al., 2007b, Driver et al., 2012, Driver et al., 2013). Hyperoxia increases arterial oxygen content (mostly through an increase in O_2 dissolved in blood plasma), thus increasing venous oxygen saturation, and hence decreasing the concentration of deoxyhaemoglobin in capillaries and veins (Rostrup et al., 1995). This leads to an increase in the transverse relaxation time (T_2^*) of blood in vessels and the surrounding tissue, and thus a global increase in the BOLD-MRI signal (Losert et al., 2002, Ogawa and Lee, 1990).

Calibrated functional magnetic resonance imaging (fMRI) provides a non-invasive method of quantifying the fractional change in cerebral metabolic rate of oxygen ($CMRO_2$) consumption giving rise to a BOLD signal change during an fMRI experiment (Kastrup et al., 2002, Chiarelli et al., 2007a, Stefanovic et al., 2005, Uludag et al., 2004, Mohtasib et al., 2012, Davis et al., 1998). Hyperoxia has been suggested as an alternative to the more common hypercapnia-based BOLD calibration (Chiarelli et al., 2007b, Driver et al., 2012), providing the advantages of a more precise estimate of $CMRO_2$ and a more tolerable stimulus which can be applied for longer periods. Furthermore, hypercapnia-based BOLD calibration is likely to be compromised by the known change in electrophysiological activity with hypercapnic stimuli, and the impact this may have on $CMRO_2$ (Jones et al., 2005, Hall et al., 2011, Xu et al., 2011, Zappe et al., 2008, Thesen et al., 2012), although such a reduction in $CMRO_2$ is contested (Chen and Pike, 2010). To date, there remains some doubt as to the effect of hyperoxia on tissue blood flow and neuronal activity, and any such changes would complicate or undermine the use of hyperoxia-based BOLD calibration. More recently, a combined method of hyperoxia-hypercapnia BOLD calibration has been proposed (Bulte et al., 2012, Gauthier and Hoge, 2012), with the potential to offer a clinically viable alternative to positron emission PET for quantification of baseline $CMRO_2$. However, this will combine

the potential pitfalls associated with the separate delivery of hyperoxia and hypercapnia, and so further emphasises the need to fully understand the accuracy of each of these methods.

Reports in the literature as to the effect of hyperoxia on tissue blood flow are contradictory (Kety and Schmidt, 1948, Watson et al., 2000, Kolbitsch et al., 2002, Bulte et al., 2007b). Some previous studies may have been confounded by associated arterial hypocapnia, caused by the Haldane effect (a reduction in CO_2 carrying capacity of oxyhaemoglobin during hyperoxia) (Becker et al., 1996, Loeppky et al., 1983). For example, Bulte et al. reported a reduction of approximately 4 mmHg in $P_{ET}CO_2$ under 60 % O_2 (Bulte et al., 2007b). Since the vasculature is very sensitive to changes in blood levels of CO_2 (Bray, 1999), a relatively low level of concomitant hypocapnia, may significantly decrease blood flow. Therefore, to properly characterise the effect of hyperoxia, it is important to control or monitor any confounding changes in the level of CO_2. An additional complication is that the technique of Arterial Spin Labelling (ASL), which is frequently used to measure cerebral blood flow (CBF), is sensitive to tissue and blood longitudinal relaxation times (T_1). During hyperoxia, dissolved plasma oxygen shortens the T_1 of blood (Noseworthy et al., 1999, Tadamura et al., 1997) and tissue (O'Connor et al., 2007) which would lead to an under-estimation of CBF during hyperoxia if not properly accounted for when modelling the data.

The effect of hyperoxia on underlying neuronal activity can also be studied using electrophysiology. Using electroencephalography (EEG) to assess the effect of 35 % O_2 on cognitive performance and brain activity, Seo et al. (2007b) found a reduction in beta and gamma power in the left and right hemispheres, an increase in delta power in the left and right hemispheres, and a reduction in alpha power in the left side of the brain. However, others (Lindauer et al., 2003, Smith and Strawbridge, 1974, Kaskinoro et al., 2010) have found no significant effect of a change in blood oxygenation level on electrophysiology signals. These studies have either employed scalp level electric field measures (EEG) or very focal invasive electrode recordings (rat whisker studies (Lindauer et al., 2003)). Magnetoencephalography (MEG) provides a non-invasive method of spatially resolving the electrophysiological effects of hyperoxia across cortical grey matter.

The aim of this study was to assess changes in blood flow and localised neuronal activity in response to isocapnic hyperoxia. We used a RespirAct^{TM} (Thornhill Research Inc., Toronto, Canada) system to provide independent control of end-tidal levels of O_2 ($P_{ET}O_2$) and CO_2

($P_{ET}CO_2$). MR measurements were performed at ultra-high field (7 T) to provide increased signal-to-noise ratio (SNR), and lengthened T_1 relaxation times, increasing the contrast-to-noise ratio (CNR) in ASL (Gardener et al., 2009). ASL measurements were used to quantify arterial cerebral blood volume (aCBV), rather than CBF, as aCBV is thought to drive the CBF and BOLD signal changes (Brookes et al., 2007, Zheng et al., 2002) and aCBV weighted ASL has greater sensitivity than CBF weighted ASL measures (Brookes et al., 2007, Zheng et al., 2002). Phase contrast (PC) MRI was used as a T_1 independent measure of blood flow. Finally, MEG was used to monitor the effects of hyperoxia on electrophysiological brain activity.

METHODS

This study comprised two MR sessions (Experiment 1 and 2) and one MEG session (Experiment 3). The study was approved by the University of Nottingham Medical School ethics committee and subjects gave prior informed written consent. Six subjects (aged 24-28 yrs, 4 female) participated in Experiment 1, 7 subjects (age 24-48 yrs, 3 female,) participated in Experiment 2, and 9 subjects (age range 23-30 years, 4 female) participated in Experiment 3. MR scanning was performed using a Philips Achieva 7.0 T system with head volume transmit and 32-ch SENSE receive coil with foam padding used to reduce head motion. MEG recordings were made using a 275-channel axial gradiometer (CTF) MEG system (MISL, Port Coquitlam, BC, Canada)

A feed-forward, low gas flow system (RespirAct™, Thornhill Research Inc., Toronto, Canada) and a sequential gas delivery (SGD) breathing circuit were used to target end-tidal PCO_2 ($P_{ET}CO_2$) and PO_2 ($P_{ET}O_2$) independently (Slessarev et al., 2007), and maintain a constant normoxic baseline where necessary Source gases used by the system were 100 % O_2, medical air and two blends of O_2, CO_2 and N_2 gas each containing a minimum of 10 % O_2 for safety purposes. Using the approach of Slessarev et al. (2007), the RespirAct™ determines the required flow of these source gases, to target pre-determined $P_{ET}CO_2$ and $P_{ET}O_2$ values. Prior to MRI or MEG measurement, the subject sat upright on the scanner bed while baseline metabolic values were estimated and targeted. Whilst lying supine in the scanner, the subject received medical air via the RespirAct™ until the respiratory challenge commenced.

Experiment 1: Phase Contrast MRI flow measurement under hyperoxia and hypercapnia. The experiment involved 1 minute of normoxic baseline followed by 5 minutes of isocapnic hyperoxia ($P_{ET}O_2$ targeted at 500mmHg, equivalent to F_iO_2 = 0.6, at subject's resting $P_{ET}CO_2$, included as a positive control), and then 5 minutes of normoxic baseline. This was then followed by 2 cycles of 2 minutes of hypercapnia (subject's resting $P_{ET}O_2$, $P_{ET}CO_2$ targeted at baseline + 8mmHg), separated by 2 minutes of baseline, followed by 1 minute of baseline (Figure 1A). The total duration of the respiratory challenge was 20 minutes, including transitions between the different blood gas levels. All hyperoxia transitions consisted of a graded increase/decrease in target $PETO_2$ (between subject specific baseline and 500mmHg across 1 minute) in order to minimise a sudden influx of gas, as is common with a square-wave transition. This minimises discrepancies between targeted and actual $PETO_2$, allowing a steady-state to be reached near instantaneously.

Sagittal and coronal 2D PC-MRA data sets (2 slices of thickness 30 mm, TR/TE = 14/7.6 ms, FA = 20°, FOV = 230 x 230 mm^2, SENSE 2, v_{ENC} = 30 cm/s scan duration = 47 s for number of signal averages (NSA) 4) were acquired prior to the respiratory challenge to locate the left and right internal carotid arteries (ICA) and other major vessels in the neck. Blood flow in each ICA was measured using a vectorcardiogram (VCG) gated, 2D PC-MRA (TR/TE = 15/6.5 ms, FA = 25°, FOV = 280 x 77 mm^2, 0.75 x 0.75 x 6 mm^3 reconstructed, SENSE 4, v_{ENC} = 0 and 100 cm/s, scan duration = 1 min 25 s for 2 averages) on a single slice perpendicular to the targeted ICA with 16-25 phases (dependent on subject heart rate). The measurement plane was positioned through the C1 segment of the spinal cord, where the left and right ICA were approximately parallel (Figure 2A). PC-MRA data were collected throughout the 20 minute respiratory challenge with 4 repeats collected at normoxia, hyperoxia, and hypercapnia.

PC-MRI flow data were analyzed using QFlow software (Philips, Best, Netherlands). Regions of interest (ROIs) were drawn manually around the lumen of the carotid arteries on each phase contrast image (Figure 2B), contour detection software was used to improve the ROI accuracy. The mean signal intensity within each ROI reflected the flow velocity in the vessel (cm/s) for each cardiac phase. The cross-sectional area of each vessel lumen was multiplied by the velocity, to compute, for each cardiac phase, the carotid artery blood flow in ml/min.

The flux data were then averaged across left and right ICAs, and repeated measurements, to give an average carotid artery flux waveform at normoxia, hyperoxia and hypercapnia. These data were then averaged across all subjects to give a mean response across cardiac cycle, and across all phases of the cardiac cycle to provide a single value from which to estimate mean blood flow (MBF) at normoxia, hyperoxia and hypercapnia. The change in MBF induced by hyperoxia and hypercapnia was then assessed using a non-parametric Wilcoxon signed rank test (SPSS 16, Chicago, IL, USA), and the % change in MBF per mmHg CO_2 caluclated for each subject and averaged. The variance between the repeat measures of MBF calculated normoxia was assessed to estimate the minimum detectable change (MDC) in MBF at a significance of $P = 0.05$.

Experiment 2: aCBV response to hyperoxia: This experiment involved 1 minute of baseline followed by 2 minutes of isocapnic hyperoxia ($P_{ET}O_2$ targeted at 500 mmHg, subject's resting $P_{ET}CO_2$) and 1 minute of baseline (Figure 1B). $P_{ET}CO_2$ was maintained at subject specific resting levels throughout. The duration of the respiratory challenge was 6 minutes, including transitions.

Flow-sensitive alternating inversion recovery (FAIR) Arterial Spin Labelling (ASL) was combined with Look-Locker (LL) sampling to map aCBV using a two compartment vascular kinetic model (Brookes et al., 2007, Francis et al., 2008). The acquisition parameters were: initial inversion delay (TI) = 150 ms, readout spacing (TA) = 100 ms, TR = 3000 ms, TE = 16 ms, 21 phases, FA = 45°, single slice EPI with an in-plane resolution of 2 x 2 mm, FOV = 192 x 192 mm, slice thickness of 3 mm. In addition, at normoxia a base magnetisation image was acquired and a series of inversion recovery EPI images (10 inversion times, 100 - 2600 ms) which were fitted for T_1. A grey matter (GM) mask was then formed by thresholding the T_1 map at $1.7 \leq T_1 \leq 2.3$ s. The mean GM aCBV and arterial transit time ($\Delta_{arterial}$) were then calculated. First aCBV weighted difference images were formed by subtraction of the label from control images (Figure 3A). The average aCBV difference signal within the GM mask for each LL-readout was then estimated, for both the normoxia and hyperoxia periods. These signal curves were then normalised by the base magnetisation blood signal from the middle cerebral artery, and the resulting normalised aCBV signal curves at normoxia and hyperoxia fitted for GM aCBV and $\Delta_{arterial}$ as described in (Brookes et al., 2007). This fitting required an estimate of T_{1blood} on normoxia and hyperoxia. The measurement of T_{1blood} at normoxia and

hyperoxia *in vivo* in humans at 7 T is difficult due to its fast flow rate and the limited coverage of the head transmit coil, therefore we assumed a T_1 of arterial blood (F_iO_2 = 0.2) at 7 T of 2200 ms ($T1_{blood}$ normoxia) (Rane and Gore, 2013, Grgac et al., 2013). Estimate $T1_{blood}$ during at 7 T required a number of extrapolations: we first used the T_1 of arterial blood at 7 T together with that at 1.5 T of 1205 ms (Noseworthy et al., 1999) to calculate the exponent in the expression of Rooney et al. (2007) relating T_1 to field strength (B_0), giving $T_1 = 839(B_0)^{0.39}$. We then used this expression to extrapolate the value of T_{1blood} = 979 ms at F_iO_2 = 1 measured at 1.5 T (Noseworthy et al., 1999) to 1792 ms at 7 T. These 7T values of T_{1blood} at F_iO_2 = 0.2 and 1, were then exponentially interpolated (Bulte et al., 2007b) to estimate the $T1_{blood}$ at 7T for F_iO_2 = 0.6 of 1986 ms (T_{1blood} hyperoxia). Significant effects of hyperoxia on mean aCBV and arterial transit time were assessed across subjects using a non-parametric Wilcoxon signed rank test.

Experiment 3: MEG response to hyperoxia: This involved 1 minute of baseline followed by 2 cycles of 5 minutes of isocapnic hyperoxia ($P_{ET}O_2$ targeted at 500mmHg, subject's resting $P_{ET}CO_2$) separated by 5 minutes of baseline with a final minute of baseline (Figure 1C). The duration of the respiratory challenge was 21 minutes, including transitions. MEG data were acquired using a 275-channel CTF MEG system (MISL, Port Coquitlam, BC, Canada), at a rate of 600 Hz, with a 150 Hz anti-aliasing hardware filter and with synthetic third order gradiometer interference suppression. Subjects were instructed to lie supine in the system and to fixate their eyes on a dot presented on a screen located ~ 40 cm in front of them. For head localisation, three electromagnetic coils were placed on the head (nasion, left preauricular and right preauricular), the position of these coils on the subject's head were measured using a 3D digitiser (*Polhemus isotrack*). These coils were energised to locate the position of the subject's head within the MEG helmet. An MPRAGE structural scan was acquired using a Philips Achieva 3 T MR scanner (1 mm^3 isotropic resolution, 256 x 256 x 160 mm FOV, TI/TE/TR = 960/3.9/8.3 ms, FA = 8°, SENSE factor = 3 and shot interval = 3 s). The locations of the fiducial markers and MEG sensors with respect to the brain anatomy were determined by matching the digitised head surface to the head surface extracted from the structural MRI.

MEG data were processed using the method described in (Hall et al., 2011) to study the effect of hypercapnia on electrophysiological signals. First, 4 dimensional/volumetric maps of the

timecourse of the change in electrical oscillatory amplitude for the theta (4 – 8 Hz), alpha (8 – 13 Hz), beta (13 – 30 Hz) and low gamma (30 – 50 Hz) bands, were derived using a Beamformer approach on a 5 mm grid (Gross et al., 2001, Robinson and Vrba, 1998, Sekihara et al., 2001, Van Drongelen et al., 1996, Van Veen et al., 1997, Brookes et al., 2004, Brookes et al., 2008) spanning the whole brain. For all voxels and frequency bands, the difference in mean amplitude envelope between the hyperoxia and normoxia time windows (discarding transition periods) was computed and normalised by the normoxic value. Statistical significance in the difference between normoxia and hyperoxia was assessed using a Monte Carlo technique (Cheyne et al., 2003, Nichols and Holmes, 2002, Brookes et al., 2010) in which 50 'fake' pseudo-T statistical images were calculated with active and control windows randomly switched. Voxels were identified as having statistically significant changes if they fell in the upper 5th percentile of T-values computed in the randomised images. Spatial maps of percentage change in amplitude were created for the alpha, beta and gamma bands and registered to MNI space to allow group averaging.

Regions of interest were probed further by assessing oscillatory activity across multiple narrow frequency bands using a beamformer approach; the 17 frequency bands used were: 1 – 4 Hz, 2 – 6 Hz, 4 – 8 Hz, 6 – 10 Hz, 8 – 13 Hz, 10 – 15 Hz, 13 – 20 Hz, 15 – 25 Hz, 20 – 30 Hz, 25 – 35 Hz, 30 – 40 Hz, 35 – 45 Hz, 40 – 50 Hz, 45 – 55 Hz, 50 – 60 Hz, 55 – 65 Hz and 60 – 80 Hz. We chose to probe three brain locations in which changes had been previously observed for hypercapnia; the occipital lobe (MNI [-8 -80 -6] mm), the right motor cortex (MNI [34 -24 54] mm) (Hall et al., 2011) and the medial frontal cortex (MNI [-2 36 32] mm), an area associated with cognitive processing in working memory (Brookes et al., 2010). Oscillatory amplitude envelopes for each frequency band were averaged across periods of hyperoxia and normoxia, and the resulting spectra averaged across subjects. Significant differences (where $p < 0.05$) between hyperoxia and normoxia were tested using a non-parametric Wilcoxon signed rank test with Bonferroni correction for multiple comparisons.

RESULTS

Experiment 1: PC-MRI flow measurement under hyperoxia and hypercapnia: One of the six subjects was excluded due to the tortuous configuration of their ICAs, preventing the imaging slice being placed perpendicular to both left and right ICA simultaneously. The mean hyperoxic step (± standard error in mean (SEM)) was 460 ± 13 mmHg, with a baseline of 111 ± 7 mmHg averaged across subjects, while $P_{ET}CO_2$ varied by less than 1 mmHg. The mean hypercapnic step was 4.2 ± 0.5 mmHg, while $P_{ET}O_2$ varied by less than 1 mmHg.

Hyperoxia did not cause a significant change in MBF (paired t-test, power = 0.8, p = 0.80), with a 95 % confidence interval of -3.2 to + 3.3 %, suggesting that less than 5 % of results would show the 4 % reduction in MBF reported in previous literature to a comparable increase in FiO_2 of 0.6 (Bulte et al., 2007b). Further, the MDC at a significance of p = 0.05, assessed from repeated measures of normoxic MBF, was found to be 3.6 %, again less than the MBF change reported previously. As expected, an increase in MBF occurred during hypercapnia compared to normoxia of 6.8 ± 1.5 % per mmHg CO_2 (power = 0.08, p = 0.03). Figure 2D shows the variation in MBF across the cardiac cycle during normoxia, hyperoxia and hypercapnia averaged across subjects.

Experiment 2: aCBV response to hyperoxia: The mean hyperoxic step (± SEM) was 486 ± 12 mmHg, with a baseline of 119 ± 1 mmHg averaged across subjects, while $P_{ET}CO_2$ varied by less than 2 mmHg. Figure 3A shows the aCBV-weighted LL-FAIR ASL data in a representative subject, from which the mean GM aCBV was measured to be 1.2 ± 0.4 % during normoxia and 1.1 ± 0.3 % during hyperoxia. This reduction was not significant (power = 0.8, p = 0.64, paired t-test), with a 95 % confidence interval of -0.21 to + 0.31 %. There was no significant change in mean arterial transit time, Δ_a which was found to be 318 ± 7 ms during normoxia and 309 ± 4 ms during hyperoxia (power = 0,8, p = 0.68, paired t-test), with a 95% confidence interval of -69 to +95 ms.

Experiment 3: MEG response to hyperoxia: The mean hyperoxic step was 471 ± 13 mmHg, with a baseline of 116 ± 3 mmHg averaged across subjects, while $P_{ET}CO_2$ varied by less than 1 mmHg. Figure 4A depicts the group averaged maps of neural oscillatory amplitude in response to hyperoxia in the alpha, beta and gamma frequency bands. For comparison, the equivalent images for a similar hypercapnia study (Hall et al., 2011) are also shown (Figure 4B). Whereas hypercapnia elicited large scale, robust changes in MEG measured oscillatory amplitude, the equivalent changes observed using hyperoxia involve small focal areas of

amplitude reduction, predominantly in occipital brain areas. Figure 4C shows the difference in oscillatory response between hypercapnia and hyperoxia, with a positive difference during hypercapnia shown in blue and during hyperoxia in red. It should be noted that regions in red are predominantly in the cerebellum, spatial locations where the SNR of MEG is limited. Maps of change in the theta band (not shown) showed no robust changes for either hyperoxia or hypercapnia.

Figures 4Di-iii show the spectral changes in neural oscillatory amplitude elicited by hyperoxia and hypercapnia (Hall et al., 2011) in three selected brain regions. Figures 4Ei-iii show the corresponding changes in amplitude across the alpha, beta and gamma band. As also seen in Figure 4A, the most pronounced change occurred in the occipital lobe (MNI [-8 -80 -6] mm) where a significant reduction in amplitude (Wilcoxon signed rank test, $p<0.05$) was observed in the 10 - 40 Hz band.

DISCUSSION

The increasing use of hyperoxia as a tool for studying cortical haemodynamics and calibrating the BOLD effect, creates an urgent need to understand the impact of hyperoxia on brain physiology. In this paper we show that isocapnic hyperoxia causes no significant effect on MBF as measured with PC-MRI or aCBV as measured with LL-FAIR ASL. We also show that hyperoxia causes a smaller and more focal change in neural oscillatory activity, as characterised by MEG, compared to hypercapnia.

MBF sensitivity to hyperoxia was measured in the carotid arteries to give maximum sensitivity to global changes in CBF. A phase contrast based MR method was used to measure MBF to ensure insensitivity to any changes in the T_1 relaxation time of the blood or tissue due to hyperoxia. Whilst measurement in the carotid arteries alone neglects the posterior contribution to global CBF, it has been shown that the anterior circulation provides up to 82 % of cortical flow (Boyajian et al., 1995, Zhao et al., 2007), with both systems reacting equally to external stimuli (Field et al., 2003). It was not possible to also collect PCA data from both the anterior and posterior circulations in a reasonable acquisition time.

We found no change in grey matter aCBV during hyperoxia. aCBV was assessed since ASL measurements of aCBV have much higher sensitivity than ASL measurements of CBF (Brookes et al., 2007, Zheng et al., 2002). Furthermore, aCBV reflects vasoconstriction of the

arterioles which controls blood flow through the capillaries and hence tissue perfusion, and the aCBV compartment is known to be more responsive to activation compared to the venous blood volume (Lee et al., 2001, Brookes et al., 2007, Kim et al., 2007). However the effect of hyperoxia on total CBV is also under debate (Kolbitsch et al. 2002). aCBV was measured using LL-FAIR ASL which is a multiphase readout ASL sequence, allowing aCBV and arterial transit time to be fitted simultaneously. This is important since a change blood flow is often also accompanied by a change in transit time, and these changes may act in opposition. Therefore if a constant transit time is assumed at a single inversion time, changes in aCBV may be masked. The reduction in T_{1blood} that occurs during hyperoxia can cause an apparent change in the ASL aCBV weighted signal in the absence of any actual change in aCBV. In this work we took account of this effect by fitting the aCBV data assuming a reduction in T_{1blood} during hyperoxia (from 2200 ms at normoxia to 1986 ms at hyperoxia), where these values were estimated by extrapolating from the literature values at other field strengths and levels of oxygenation.

To investigate the effect of neglecting such a reduction in T_{1blood} on the fitted value of aCBV, the aCBV weighted signal was simulated using the LL-FAIR ASL sequence timings used in this experiment (Brookes et al., 2007), with additional simulation parameters given in Table 2. Simulating aCBV weighted LL-FAIR ASL data (100 iterations, SNR = 100:1) with T_{1blood} of 1892 ms (for hyperoxia) and fitting the data assuming $T1_{blood}$ of 2200 ms (normoxia value) led to aCBV being underestimated by 10 ± 0.3 % (error in fitted mean ± SEM across iterations), while $\Delta_{arterial}$ remained largely unaffected (underestimated by 0.6 ± 0.5 %). Maleki et al. (2011) similarly showed that CBF changes would be underestimated if the effect of hyperoxygenation on blood T_1 was ignored and a further study showed no change in CBF when correcting for the arterial blood T_1 decrease (Zaharchuk et al., 2008, Maleki et al., 2011). The aCBV signal is derived solely from the arterial blood compartment due to the suppression of the tissue signal by the rapid sequence of high flip angle pulses, and therefore it is insensitive to any changes in tissue T_1 which in any case are likely to be small in the brain. Although this simulation was carried out for aCBV weighted ASL data, these results highlight the more general importance of accounting for any change in T_{1blood} when fitting ASL signal curves for both aCBV and CBF quantification.

The PC-MRI blood flow results of a 6.8 ± 1.5 % per mmHg CO_2 change in response to hypercapnia are in line with previous literature (Mark et al., 2011, Davis et al., 1998, Bulte et

al., 2009, Ito et al., 2003, Rostrup et al., 2000). The hyperoxia results presented here support the findings of a recent ASL study (Mark et al., 2011) which found no significant change in GM CBF in response to isocapnic hyperoxia. However, that ASL study did not correct for a change in T_1 blood relaxation time which would have led to an underestimation of CBF, potentially masking an actual increase CBF. It is likely that previously reported reductions in CBF during hyperoxia measured with PC MRI can be attributed to the hypocapnia accompanying the hyperoxia since CO_2 changes were not controlled (Rostrup et al., 1995, Watson et al., 2000). It should be noted that the MBF and aCBV measures were from a modest sample size in comparison to previous studies. However, previous reports of vasoconstrictive effects of hyperoxia (Bulte et al., 2007b, Watson et al., 2000, Rostrup et al., 2000, Rostrup et al., 1995) fall outside of the 95% confidence interval for our reported measures of both MBF and aCBV, therefore it is likely that a change of such magnitude would have been measured within this experimental design.

This study also found that hyperoxia produces small focal changes in spontaneous MEG signals. This was compared to previous studies that reported that hypercapnia had a global effect of greater amplitude (Hall et al., 2011, Thesen et al., 2012). Both of these findings could significantly impact on the fundamental assumptions regarding the isometabolic nature of hyperoxia and hypercapnia for calibrated BOLD, albeit hyperoxia to a lesser extent. Although there is no direct causal link between neuronal oscillations and changes in $CMRO_2$, it is believed that MEG signals are generated by synchronised, post-synaptic current flow in the dendrites of pyramidal neurons, and theoretical evidence (Attwell and Laughlin, 2001) suggests that the majority of energy use in the brain is involved with similar post synaptic events. This suggests that a change in the MEG signal is indirectly associated with energy demand. This has been supported by invasive studies in animals (Logothetis et al., 2001) and humans (Mukamel et al., 2005, Ossandon et al., 2011, Ossandon et al., 2012), as well as non-invasive studies using MEG (Brookes et al., 2005, Singh et al., 2003, Zumer et al., 2010, Stevenson et al., 2011, Winterer et al., 2007) which suggest a relationship between task driven modulations of neural oscillations and the BOLD response. Furthermore recent evidence (Murphy et al., 2013) shows that hypercapnia induces measurable, spatially-dependent changes in $CMRO_2$ which are in good agreement with the electrophysiological oscillatory changes previously reported (Hall et al., 2011). Therefore it seems unlikely that the changes in neural oscillations observed in Figures 4 and 5 can occur without concomitant changes in cerebral energetics.

Alpha activity is often most prominent in the occipital lobe, where it is thought to reflect activity of the thalamo-cortical loop; increasing in an eyes-closed wakeful resting state and decreasing when the eyes are opened (Berger, 1929). The significant reduction in amplitude of low frequency neural oscillations in the occipital lobe is consistent with the reduction in alpha power in previous EEG recordings (Seo et al., 2007a). Traditionally, alpha activity was thought to reflect cortical idling (Lopes da Silva et al., 1973, Berger, 1929, Niedermeyer and Lopes da Silva, 1999), more recently, this has been updated to include the notion that increased alpha activity represents increased functional inhibition (Jensen and Mazaheri, 2010, Jensen et al., 2012, Klimesch et al., 2007, Thut et al., 2006). The reduction in alpha in response to hyperoxia in the occipital lobe may arise due to the greater sensitivity to alpha power in this area, or reflect an increase in vigilance with supplementary O_2 (Moss et al., 1998). Hyperoxia has been shown to enhance cognitive function to include long term memory (Moss et al., 1998), working memory and word recall . The dynamic relationship between altered vigilance, task performance and cerebral metabolism under hyperoxia needs further investigation. Confounding effects due to patient discomfort and somatosensory activation are reduced as it is difficult to perceive the difference between inspiring high levels of oxygen required for hyperoxia and medical air, as highlighted by its use in placebo-controlled trials (Ozkurt et al., 2012, Moss et al., 1998, Cohen et al., 2009).

These results have important implications for calibrated BOLD measurements using hyperoxia (Chiarelli et al., 2007b, Goodwin et al., 2009, Driver et al., 2012), and the more recent combined O_2 and CO_2 calibrated BOLD methods (Gauthier and Hoge, 2012, Bulte et al., 2012). The results imply that hyperoxia based methods of BOLD calibration need not be corrected for changes in baseline CBF or CBV (Chiarelli et al., 2007b) (hypercapnia based BOLD calibration does not require any such correction since changes in CBF on hypercapnia are implicit in the method). Hyperoxia can also be used to investigate vascular function and structure, as highlighted by its recent use in venous CBV estimations (Blockley et al., 2012) and vessel size imaging (Shen et al., 2011), techniques for which the lack of an effect of hyperoxia on blood flow is important.

The altered neural oscillatory processes induced by both hyperoxia and hypercapnia (Hall et al., 2011) will indeed impact on the assumption of iso-metabolism made in BOLD calibration. However, we find that isocapnic hyperoxia induces neural oscillatory changes

that are smaller in magnitude and spatial extent across subjects compared to iso-oxic hypercapnia which implies that isocapnic hyperoxia is a more appropriate stimulus than hypercapnia for calibrating the BOLD response (Chiarelli et al., 2007b). This said, the small focal effect of hyperoxia on neural signals remains significant, suggesting that further investigation is required in order to determine if the observed change may lead to an under/overestimation of $CMRO_2$ in these areas. The significant but differential effects of hyperoxia and hypercapnia on neural oscillatory processes may be of even greater importance to the more recent combined calibrated BOLD models (Bulte et al., 2012, Gauthier and Hoge, 2012). Further investigation would allow quantification of separate hyperoxia and hypercapnia $CMRO_2$ error terms in order to gain equivalent calibration constants which can then be combined

CONCLUSION

This study addresses the main physiological assumptions made in hyperoxia-based calibrated BOLD, and has found no significant effect of isocapnic hyperoxia on GM aCBV or global MBF in the internal carotid arteries. While hyperoxia was shown to produce significant changes in neuronal oscillations consistent with increased vigilance as measured using source localised MEG, the changes were both more focal and of significantly smaller amplitude than observed with hypercapnia (Hall et al., 2011). This supports the use of hyperoxia in preference to hypercapnia for calibrating the BOLD response (Chiarelli et al., 2007b), however further investigation is needed on the impact of changes in neuronal oscillations on $CMRO_2$ consumption and the precision of combined hyperoxia-hypercapnia calibration.

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TABLES:

Table 1: Effect of hyperoxia and hypercapnia compared to normoxia (mean ± SEM) on mean blood flow (MBF) averaged over left and right ICA, mean aCBV, arterial transit time ($\Delta_{arterial}$). Data averaged across all subjects. * denotes a significant change where $p<0.05$.

Table 2: Parameters used to simulate aCBV data (Brookes et al. 2007; Francis et al. 2008)

Table 1

	Normoxia	Hyperoxia	Hypercapnia
MBF (ml/min)	201 ± 11	201 ± 12	272 ± 14*
aCBV	1.3 ± 0.4	1.2 ± 0.3	--
$\Delta_{arterial}$ (ms)	0.32 ± 0.07	0.31 ± 0.04	--

Table 2

Parameter	**Value**
Blood volume, aCBV (%) (assumed using a mean arterial flow rate F of 180 ml/100g and exchange time, Δ, of 800 ms) (model described in (Brookes et al., 2007, Zheng et al., 2002).	2.4
Longitudinal relaxation time of tissue, $T_{1tissue}$ (ms) ((Rooney et al., 2007)	2200
Arterial transit time, $\Delta_{arterial}$ (ms)	100
Bolus duration, W (ms)	1200
Equilibrium magnetisation, M_0	1
Simulated T_{1blood} (ms) at 7T for FiO2 (%) = 0.2, 0.4, 0.6, 0.8, 1.0 (Extrapolated from (Rooney et al., 2007) and (Bulte et al. 2007b))	2200, 2144 2090, 2037, 1986

FIGURES

Figure 1: Targeted respiratory paradigms for A) Experiment 1; isocapnic hyperoxia ($P_{ET}O_2$ = 500 mmHg) followed by 2 cycles of iso-oxic hypercapnia (subject specific baseline $P_{ET}CO_2$ + 8 mmHg) B) Experiment 2; isocapnic hyperoxia ($P_{ET}O_2$ = 500 mmHg), $P_{ET}CO_2$ targeted at subject-specific baseline throughout and C) Experiment 3; 2 cycles of isocapnic hyperoxia ($P_{ET}O_2$ = 500 mmHg), $P_{ET}CO_2$ targeted at subject-specific baseline throughout. Requested end tidal pressure of O_2 is traced in black and CO_2 in grey.

Figure 2: A) 2D sagittal phase-contrast angiogram for localisation of the 2D PC-MR imaging slice (dashed white line) perpendicular to left and right internal carotid arteries, and 2D PC-MRI modulus B) and phase C) images. D) The effect of normoxia (grey solid line), hyperoxia (black solid line) and hypercapnia (black dashed line) on the mean blood flow (MBF) waveform averaged across subjects. Averaging over the left and right ICA and 16 cardiac phases provides an MBF of 201 ml/min for normoxia, 201 ml/min for hyperoxia and 272 ml/min for hypercapnia, whilst the peak of the waveform provides peak blood flow (268 ml/min for normoxia, 254 ml/min for hyperoxia and 391 ml/min for hypercapnia). Error bars indicate the standard error of mean.

Figure 3: A) Example perfusion weighted LL-FAIR aCBV data. The effective post label delay is given in milliseconds. B) GM aCBV weighted normalised LL ASL signal curves at normoxia (grey solid line) and hyperoxia (black solid line). Data averaged across subjects. Error bars indicate the standard error of mean.

Figure 4: A) Group maps of the significant reductions in neural oscillatory amplitude in response to hyperoxia (3-15%), in the alpha (8-13Hz), beta (13-30 Hz) and gamma (30-50 Hz) bands. B) This is contrasted with previously reported reductions from a hypercapnic stimulus (3-15 %) (Hall et al. 2011) which produced a robust global desynchronisation, evident across all subjects. C) The regions where a hypercapnia induced change is greater than a hyperoxia induced change are shown in dark/light blue (0.5-7%). Regions where a hyperoxia induced change are greater are shown in red/yellow (0.5-7%). In the hyperoxic case, changes are smaller in magnitude and spatial extent, and are less robust across subjects. The reduction in neural oscillatory power (mean ± SEM) was interrogated across D)i) the motor cortex, ii) the occipital lobe and iii) the medial frontal cortex in response to hyperoxia (black) and

hypercapnia (grey) (Hall et al. 2011). E)i-iii) This power change is further compared in the same regions across the alpha, beta and gamma band.