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

2

3 **Paula L. Croal, Emma L. Hall, Ian D. Driver, Matthew J. Brookes, Penny A. Gowland,**
4 **Susan T. Francis ***

5

6 Sir Peter Mansfield Magnetic Resonance Centre, School of Physics and Astronomy,
7 University of Nottingham, UK.

8

9 *Corresponding Author:

10 Sir Peter Mansfield Magnetic Resonance Centre

11 School of Physics and Astronomy

12 University of Nottingham

13 Nottingham

14 NG7 2RD

15 Email: susan.francis@nottingham.ac.uk

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1 **ABSTRACT**

2 The physiological effect of hyperoxia has been poorly characterised, with studies reporting
3 conflicting results on the role of hyperoxia as a vasoconstrictor. It is not clear whether
4 hyperoxia is the primary contributor to vasoconstriction or whether induced changes in CO₂
5 that commonly accompany hyperoxia are a factor. As calibrated BOLD fMRI based on
6 hyperoxia becomes more widely used, it is essential to understand the effects of oxygen on
7 resting cerebral physiology. This study used a RespirAct™ system to deliver a repeatable
8 isocapnic hyperoxia stimulus to investigate the independent effect of O₂ on cerebral
9 physiology, removing any potential confounds related to altered CO₂. T₁-independent Phase
10 Contrast MRI was used to demonstrate that isocapnic hyperoxia has no significant effect on
11 carotid blood flow (normoxia 201 ± 11 ml/min, -0.3 ± 0.8 % change during hyperoxia, p =
12 0.8), whilst Look Locker ASL was used to demonstrate that there is no significant change in
13 arterial cerebral blood volume (normoxia 1.3 ± 0.4 %, -0.5 ± 5 % change during hyperoxia).
14 These are in contrast to significant changes in blood flow observed for hypercapnia (6.8 ±
15 1.5 %/mmHg CO₂). In addition, magnetoencephalography provided a method to monitor the
16 effect of isocapnic hyperoxia on neuronal oscillatory power. In response to hyperoxia, a
17 significant focal decrease in oscillatory power was observed across the alpha, beta and low
18 gamma bands in the occipital lobe, compared to a more global significant decrease on
19 hypercapnia. This work suggests that isocapnic hyperoxia provides a more reliable stimulus
20 than hypercapnia for calibrated BOLD, and that previous reports of vasoconstriction during
21 hyperoxia probably reflect the effects of hyperoxia-induced changes in CO₂. However,
22 hyperoxia does induce changes in oscillatory power consistent with an increase in vigilance,
23 but these changes are smaller than those observed under hypercapnia. The effect of this
24 change in neural activity on calibrated BOLD using hyperoxia or combined hyperoxia and
25 hypercapnia needs further investigation.

26

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

29

1 **INTRODUCTION**

2 Hyperoxia (raising the inspired fraction of oxygen (FiO_2) above normal physiological levels
3 (0.21)), is increasingly being used to provide exogenous contrast in functional magnetic
4 resonance imaging (fMRI), primarily to provide a method of calibrating the blood
5 oxygenation level dependent (BOLD) effect, but also to study venous blood oxygenation and
6 venous blood volume (Blockley et al., 2012, Kwong et al., 1995, Rostrup et al., 1995, Bulte
7 et al., 2007a, Bulte et al., 2007b, Chiarelli et al., 2007a, Chiarelli et al., 2007b, Driver et al.,
8 2012, Driver et al., 2013). Hyperoxia increases arterial oxygen content (mostly through an
9 increase in O_2 dissolved in blood plasma), thus increasing venous oxygen saturation, and
10 hence decreasing the concentration of deoxyhaemoglobin in capillaries and veins (Rostrup et
11 al., 1995). This leads to an increase in the transverse relaxation time (T_2^*) of blood in vessels
12 and the surrounding tissue, and thus a global increase in the BOLD-MRI signal (Losert et al.,
13 2002, Ogawa and Lee, 1990).

14
15 Calibrated functional magnetic resonance imaging (fMRI) provides a non-invasive method of
16 quantifying the fractional change in cerebral metabolic rate of oxygen (CMRO_2) consumption
17 giving rise to a BOLD signal change during an fMRI experiment (Kastrup et al., 2002,
18 Chiarelli et al., 2007a, Stefanovic et al., 2005, Uludag et al., 2004, Mohtasib et al., 2012,
19 Davis et al., 1998). Hyperoxia has been suggested as an alternative to the more common
20 hypercapnia-based BOLD calibration (Chiarelli et al., 2007b, Driver et al., 2012), providing
21 the advantages of a more precise estimate of CMRO_2 and a more tolerable stimulus which
22 can be applied for longer periods. Furthermore, hypercapnia-based BOLD calibration is
23 likely to be compromised by the known change in electrophysiological activity with
24 hypercapnic stimuli, and the impact this may have on CMRO_2 (Jones et al., 2005, Hall et al.,
25 2011, Xu et al., 2011, Zappe et al., 2008, Thesen et al., 2012), although such a reduction in
26 CMRO_2 is contested (Chen and Pike, 2010). To date, there remains some doubt as to the
27 effect of hyperoxia on tissue blood flow and neuronal activity, and any such changes would
28 complicate or undermine the use of hyperoxia-based BOLD calibration. More recently, a
29 combined method of hyperoxia-hypercapnia BOLD calibration has been proposed (Bulte et
30 al., 2012, Gauthier and Hoge, 2012), with the potential to offer a clinically viable alternative
31 to positron emission PET for quantification of baseline CMRO_2 . However, this will combine

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

3
4 Reports in the literature as to the effect of hyperoxia on tissue blood flow are contradictory
5 (Kety and Schmidt, 1948, Watson et al., 2000, Kolbitsch et al., 2002, Bulte et al., 2007b).
6 Some previous studies may have been confounded by associated arterial hypocapnia, caused
7 by the Haldane effect (a reduction in CO₂ carrying capacity of oxyhaemoglobin during
8 hyperoxia) (Becker et al., 1996, Loeppky et al., 1983). For example, Bulte et al. reported a
9 reduction of approximately 4 mmHg in P_{ET}CO₂ under 60 % O₂ (Bulte et al., 2007b). Since
10 the vasculature is very sensitive to changes in blood levels of CO₂ (Bray, 1999), a relatively
11 low level of concomitant hypocapnia, may significantly decrease blood flow. Therefore, to
12 properly characterise the effect of hyperoxia, it is important to control or monitor any
13 confounding changes in the level of CO₂. An additional complication is that the technique of
14 Arterial Spin Labelling (ASL), which is frequently used to measure cerebral blood flow
15 (CBF), is sensitive to tissue and blood longitudinal relaxation times (T₁). During hyperoxia,
16 dissolved plasma oxygen shortens the T₁ of blood (Noseworthy et al., 1999, Tadamura et al.,
17 1997) and tissue (O'Connor et al., 2007) which would lead to an under-estimation of CBF
18 during hyperoxia if not properly accounted for when modelling the data.

19
20 The effect of hyperoxia on underlying neuronal activity can also be studied using
21 electrophysiology. Using electroencephalography (EEG) to assess the effect of 35 % O₂ on
22 cognitive performance and brain activity, Seo et al. (2007b) found a reduction in beta and
23 gamma power in the left and right hemispheres, an increase in delta power in the left and
24 right hemispheres, and a reduction in alpha power in the left side of the brain. However,
25 others (Lindauer et al., 2003, Smith and Strawbridge, 1974, Kaskinoro et al., 2010) have
26 found no significant effect of a change in blood oxygenation level on electrophysiology
27 signals. These studies have either employed scalp level electric field measures (EEG) or very
28 focal invasive electrode recordings (rat whisker studies (Lindauer et al., 2003)).
29 Magnetoencephalography (MEG) provides a non-invasive method of spatially resolving the
30 electrophysiological effects of hyperoxia across cortical grey matter.

31
32 The aim of this study was to assess changes in blood flow and localised neuronal activity in
33 response to isocapnic hyperoxia. We used a RespirAct™ (Thornhill Research Inc., Toronto,
34 Canada) system to provide independent control of end-tidal levels of O₂ (P_{ET}O₂) and CO₂

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

11 **METHODS**

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

21
22 A feed-forward, low gas flow system (RespirAct™, Thornhill Research Inc., Toronto,
23 Canada) and a sequential gas delivery (SGD) breathing circuit were used to target end-tidal
24 PCO_2 ($P_{ET}CO_2$) and PO_2 ($P_{ET}O_2$) independently (Slessarev et al., 2007), and maintain a
25 constant normoxic baseline where necessary Source gases used by the system were 100 %
26 O_2 , medical air and two blends of O_2 , CO_2 and N_2 gas each containing a minimum of 10 % O_2
27 for safety purposes. Using the approach of Slessarev et al. (2007), the RespirAct™
28 determines the required flow of these source gases, to target pre-determined $P_{ET}CO_2$ and
29 $P_{ET}O_2$ values. Prior to MRI or MEG measurement, the subject sat upright on the scanner bed
30 while baseline metabolic values were estimated and targeted. Whilst lying supine in the
31 scanner, the subject received medical air via the RespirAct™ until the respiratory challenge
32 commenced.

1
2 Experiment 1: Phase Contrast MRI flow measurement under hyperoxia and hypercapnia. The
3 experiment involved 1 minute of normoxic baseline followed by 5 minutes of isocapnic
4 hyperoxia ($P_{ET}O_2$ targeted at 500mmHg, equivalent to $F_iO_2 = 0.6$, at subject's resting
5 $P_{ET}CO_2$, included as a positive control), and then 5 minutes of normoxic baseline. This was
6 then followed by 2 cycles of 2 minutes of hypercapnia (subject's resting $P_{ET}O_2$, $P_{ET}CO_2$
7 targeted at baseline + 8mmHg), separated by 2 minutes of baseline, followed by 1 minute of
8 baseline (Figure 1A). The total duration of the respiratory challenge was 20 minutes,
9 including transitions between the different blood gas levels. All hyperoxia transitions
10 consisted of a graded increase/decrease in target $P_{ET}O_2$ (between subject specific baseline
11 and 500mmHg across 1 minute) in order to minimise a sudden influx of gas, as is common
12 with a square-wave transition. This minimises discrepancies between targeted and actual
13 $P_{ET}O_2$, allowing a steady-state to be reached near instantaneously.

14
15 Sagittal and coronal 2D PC-MRA data sets (2 slices of thickness 30 mm, TR/TE = 14/7.6 ms,
16 FA = 20°, FOV = 230 x 230 mm², SENSE 2, $v_{ENC} = 30$ cm/s scan duration = 47 s for number
17 of signal averages (NSA) 4) were acquired prior to the respiratory challenge to locate the left
18 and right internal carotid arteries (ICA) and other major vessels in the neck. Blood flow in
19 each ICA was measured using a vectorcardiogram (VCG) gated, 2D PC-MRA (TR/TE =
20 15/6.5 ms, FA = 25°, FOV = 280 x 77 mm², 0.75 x 0.75 x 6 mm³ reconstructed, SENSE 4,
21 $v_{ENC} = 0$ and 100 cm/s, scan duration = 1 min 25 s for 2 averages) on a single slice
22 perpendicular to the targeted ICA with 16-25 phases (dependent on subject heart rate). The
23 measurement plane was positioned through the C1 segment of the spinal cord, where the left
24 and right ICA were approximately parallel (Figure 2A). PC-MRA data were collected
25 throughout the 20 minute respiratory challenge with 4 repeats collected at normoxia,
26 hyperoxia, and hypercapnia.

27
28 PC-MRI flow data were analyzed using QFlow software (Philips, Best, Netherlands).
29 Regions of interest (ROIs) were drawn manually around the lumen of the carotid arteries on
30 each phase contrast image (Figure 2B), contour detection software was used to improve the
31 ROI accuracy. The mean signal intensity within each ROI reflected the flow velocity in the
32 vessel (cm/s) for each cardiac phase. The cross-sectional area of each vessel lumen was
33 multiplied by the velocity, to compute, for each cardiac phase, the carotid artery blood flow
34 in ml/min.

1

2 The flux data were then averaged across left and right ICAs, and repeated measurements, to
3 give an average carotid artery flux waveform at normoxia, hyperoxia and hypercapnia. These
4 data were then averaged across all subjects to give a mean response across cardiac cycle, and
5 across all phases of the cardiac cycle to provide a single value from which to estimate mean
6 blood flow (MBF) at normoxia, hyperoxia and hypercapnia. The change in MBF induced by
7 hyperoxia and hypercapnia was then assessed using a non-parametric Wilcoxon signed rank
8 test (SPSS 16, Chicago, IL, USA), and the % change in MBF per mmHg CO₂ calculated for
9 each subject and averaged. The variance between the repeat measures of MBF calculated
10 normoxia was assessed to estimate the minimum detectable change (MDC) in MBF at a
11 significance of $P = 0.05$.

12

13 Experiment 2: aCBV response to hyperoxia: This experiment involved 1 minute of baseline
14 followed by 2 minutes of isocapnic hyperoxia ($P_{ET}O_2$ targeted at 500 mmHg, subject's resting
15 $P_{ET}CO_2$) and 1 minute of baseline (Figure 1B). $P_{ET}CO_2$ was maintained at subject specific
16 resting levels throughout. The duration of the respiratory challenge was 6 minutes, including
17 transitions.

18

19 Flow-sensitive alternating inversion recovery (FAIR) Arterial Spin Labelling (ASL) was
20 combined with Look-Locker (LL) sampling to map aCBV using a two compartment vascular
21 kinetic model (Brookes et al., 2007, Francis et al., 2008). The acquisition parameters were:
22 initial inversion delay (TI) = 150 ms, readout spacing (TA) = 100 ms, TR = 3000 ms, TE =
23 16 ms, 21 phases, FA = 45°, single slice EPI with an in-plane resolution of 2 x 2 mm, FOV =
24 192 x 192 mm, slice thickness of 3 mm. In addition, at normoxia a base magnetisation image
25 was acquired and a series of inversion recovery EPI images (10 inversion times, 100 - 2600
26 ms) which were fitted for T_1 . A grey matter (GM) mask was then formed by thresholding the
27 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
28 calculated. First aCBV weighted difference images were formed by subtraction of the label
29 from control images (Figure 3A). The average aCBV difference signal within the GM mask
30 for each LL-readout was then estimated, for both the normoxia and hyperoxia periods. These
31 signal curves were then normalised by the base magnetisation blood signal from the middle
32 cerebral artery, and the resulting normalised aCBV signal curves at normoxia and hyperoxia
33 fitted for GM aCBV and $\Delta_{arterial}$ as described in (Brookes et al., 2007). This fitting required an
34 estimate of T_{1blood} on normoxia and hyperoxia. The measurement of T_{1blood} at normoxia and

1 hyperoxia in vivo in humans at 7 T is difficult due to its fast flow rate and the limited
2 coverage of the head transmit coil, therefore we assumed a T_1 of arterial blood ($F_iO_2 = 0.2$) at
3 7 T of 2200 ms ($T_{1\text{blood}}$ normoxia) (Rane and Gore, 2013, Grgac et al., 2013). Estimate
4 $T_{1\text{blood}}$ during at 7 T required a number of extrapolations: we first used the T_1 of arterial
5 blood at 7 T together with that at 1.5 T of 1205 ms (Noseworthy et al., 1999) to calculate the
6 exponent in the expression of Rooney et al. (2007) relating T_1 to field strength (B_0), giving T_1
7 $= 839(B_0)^{0.39}$. We then used this expression to extrapolate the value of $T_{1\text{blood}} = 979$ ms at
8 $F_iO_2 = 1$ measured at 1.5 T (Noseworthy et al., 1999) to 1792 ms at 7 T. These 7T values of
9 $T_{1\text{blood}}$ at $F_iO_2 = 0.2$ and 1, were then exponentially interpolated (Bulte et al., 2007b) to
10 estimate the $T_{1\text{blood}}$ at 7T for $F_iO_2 = 0.6$ of 1986 ms ($T_{1\text{blood}}$ hyperoxia). Significant effects of
11 hyperoxia on mean aCBV and arterial transit time were assessed across subjects using a non-
12 parametric Wilcoxon signed rank test.

13

14 Experiment 3: MEG response to hyperoxia: This involved 1 minute of baseline followed by 2
15 cycles of 5 minutes of isocapnic hyperoxia ($P_{ET}O_2$ targeted at 500mmHg, subject's resting
16 $P_{ET}CO_2$) separated by 5 minutes of baseline with a final minute of baseline (Figure 1C). The
17 duration of the respiratory challenge was 21 minutes, including transitions. MEG data were
18 acquired using a 275-channel CTF MEG system (MISL, Port Coquitlam, BC, Canada), at a
19 rate of 600 Hz, with a 150 Hz anti-aliasing hardware filter and with synthetic third order
20 gradiometer interference suppression. Subjects were instructed to lie supine in the system and
21 to fixate their eyes on a dot presented on a screen located ~ 40 cm in front of them. For head
22 localisation, three electromagnetic coils were placed on the head (nasion, left preauricular and
23 right preauricular), the position of these coils on the subject's head were measured using a 3D
24 digitiser (Polhemus isotrack). These coils were energised to locate the position of the
25 subject's head within the MEG helmet. An MPRAGE structural scan was acquired using a
26 Philips Achieva 3 T MR scanner (1 mm³ isotropic resolution, 256 x 256 x 160 mm FOV,
27 TI/TE/TR = 960/3.9/8.3 ms, FA = 8°, SENSE factor = 3 and shot interval = 3 s). The
28 locations of the fiducial markers and MEG sensors with respect to the brain anatomy were
29 determined by matching the digitised head surface to the head surface extracted from the
30 structural MRI.

31

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

1 timecourse of the change in electrical oscillatory amplitude for the theta (4 – 8 Hz), alpha (8 –
2 13 Hz), beta (13 – 30 Hz) and low gamma (30 – 50 Hz) bands, were derived using a
3 Beamformer approach on a 5 mm grid (Gross et al., 2001, Robinson and Vrba, 1998,
4 Sekihara et al., 2001, Van Dronghelen et al., 1996, Van Veen et al., 1997, Brookes et al., 2004,
5 Brookes et al., 2008) spanning the whole brain. For all voxels and frequency bands, the
6 difference in mean amplitude envelope between the hyperoxia and normoxia time windows
7 (discarding transition periods) was computed and normalised by the normoxic value.
8 Statistical significance in the difference between normoxia and hyperoxia was assessed using
9 a Monte Carlo technique (Cheyne et al., 2003, Nichols and Holmes, 2002, Brookes et al.,
10 2010) in which 50 ‘fake’ pseudo-T statistical images were calculated with active and control
11 windows randomly switched. Voxels were identified as having statistically significant
12 changes if they fell in the upper 5th percentile of T-values computed in the randomised
13 images. Spatial maps of percentage change in amplitude were created for the alpha, beta and
14 gamma bands and registered to MNI space to allow group averaging.

15

16 Regions of interest were probed further by assessing oscillatory activity across multiple
17 narrow frequency bands using a beamformer approach; the 17 frequency bands used were: 1
18 – 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
19 Hz, 25 – 35 Hz, 30 – 40 Hz, 35 – 45 Hz, 40 – 50 Hz, 45 – 55 Hz, 50 – 60 Hz, 55 – 65 Hz and
20 60 – 80 Hz. We chose to probe three brain locations in which changes had been previously
21 observed for hypercapnia; the occipital lobe (MNI [-8 -80 -6] mm), the right motor cortex
22 (MNI [34 -24 54] mm) (Hall et al., 2011) and the medial frontal cortex (MNI [-2 36 32] mm),
23 an area associated with cognitive processing in working memory (Brookes et al., 2010).
24 Oscillatory amplitude envelopes for each frequency band were averaged across periods of
25 hyperoxia and normoxia, and the resulting spectra averaged across subjects. Significant
26 differences (where $p < 0.05$) between hyperoxia and normoxia were tested using a non-
27 parametric Wilcoxon signed rank test with Bonferroni correction for multiple comparisons.

1 **RESULTS**

2 Experiment 1: PC-MRI flow measurement under hyperoxia and hypercapnia: One of the six
3 subjects was excluded due to the tortuous configuration of their ICAs, preventing the imaging
4 slice being placed perpendicular to both left and right ICA simultaneously. The mean
5 hyperoxic step (\pm standard error in mean (SEM)) was 460 ± 13 mmHg, with a baseline of 111
6 ± 7 mmHg averaged across subjects, while $P_{ET}CO_2$ varied by less than 1 mmHg. The mean
7 hypercapnic step was 4.2 ± 0.5 mmHg, while $P_{ET}O_2$ varied by less than 1 mmHg.

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

17
18 Experiment 2: aCBV response to hyperoxia: The mean hyperoxic step (\pm SEM) was 486 ± 12
19 mmHg, with a baseline of 119 ± 1 mmHg averaged across subjects, while $P_{ET}CO_2$ varied by
20 less than 2 mmHg. Figure 3A shows the aCBV-weighted LL-FAIR ASL data in a
21 representative subject, from which the mean GM aCBV was measured to be 1.2 ± 0.4 %
22 during normoxia and 1.1 ± 0.3 % during hyperoxia. This reduction was not significant (power
23 = 0.8, $p = 0.64$, paired t-test), with a 95 % confidence interval of -0.21 to + 0.31 %. There
24 was no significant change in mean arterial transit time, Δ_a which was found to be 318 ± 7 ms
25 during normoxia and 309 ± 4 ms during hyperoxia (power = 0,8, $p = 0.68$, paired t-test), with
26 a 95% confidence interval of -69 to +95 ms.

27
28 Experiment 3: MEG response to hyperoxia: The mean hyperoxic step was 471 ± 13 mmHg,
29 with a baseline of 116 ± 3 mmHg averaged across subjects, while $P_{ET}CO_2$ varied by less than
30 1 mmHg. Figure 4A depicts the group averaged maps of neural oscillatory amplitude in
31 response to hyperoxia in the alpha, beta and gamma frequency bands. For comparison, the
32 equivalent images for a similar hypercapnia study (Hall et al., 2011) are also shown (Figure
33 4B). Whereas hypercapnia elicited large scale, robust changes in MEG measured oscillatory
34 amplitude, the equivalent changes observed using hyperoxia involve small focal areas of

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

7
8 Figures 4Di-iii show the spectral changes in neural oscillatory amplitude elicited by
9 hyperoxia and hypercapnia (Hall et al., 2011) in three selected brain regions. Figures 4Ei-iii
10 show the corresponding changes in amplitude across the alpha, beta and gamma band. As
11 also seen in Figure 4A, the most pronounced change occurred in the occipital lobe (MNI [-8 -
12 80 -6] mm) where a significant reduction in amplitude (Wilcoxon signed rank test, $p < 0.05$)
13 was observed in the 10 - 40 Hz band.

14

15 **DISCUSSION**

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

22

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

31

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

1 arterioles which controls blood flow through the capillaries and hence tissue perfusion, and
2 the aCBV compartment is known to be more responsive to activation compared to the venous
3 blood volume (Lee et al., 2001, Brookes et al., 2007, Kim et al., 2007). However the effect of
4 hyperoxia on total CBV is also under debate (Kolbitsch et al. 2002). aCBV was measured
5 using LL-FAIR ASL which is a multiphase readout ASL sequence, allowing aCBV and
6 arterial transit time to be fitted simultaneously. This is important since a change blood flow is
7 often also accompanied by a change in transit time, and these changes may act in opposition.
8 Therefore if a constant transit time is assumed at a single inversion time, changes in aCBV
9 may be masked. The reduction in $T_{1\text{blood}}$ that occurs during hyperoxia can cause an apparent
10 change in the ASL aCBV weighted signal in the absence of any actual change in aCBV. In
11 this work we took account of this effect by fitting the aCBV data assuming a reduction in
12 $T_{1\text{blood}}$ during hyperoxia (from 2200 ms at normoxia to 1986 ms at hyperoxia), where these
13 values were estimated by extrapolating from the literature values at other field strengths and
14 levels of oxygenation.

15

16 To investigate the effect of neglecting such a reduction in $T_{1\text{blood}}$ on the fitted value of aCBV,
17 the aCBV weighted signal was simulated using the LL-FAIR ASL sequence timings used in
18 this experiment (Brookes et al., 2007), with additional simulation parameters given in Table
19 2. Simulating aCBV weighted LL-FAIR ASL data (100 iterations, SNR = 100:1) with $T_{1\text{blood}}$
20 of 1892 ms (for hyperoxia) and fitting the data assuming $T_{1\text{blood}}$ of 2200 ms (normoxia value)
21 led to aCBV being underestimated by $10 \pm 0.3 \%$ (error in fitted mean \pm SEM across
22 iterations), while Δ_{arterial} remained largely unaffected (underestimated by $0.6 \pm 0.5 \%$). Maleki
23 et al. (2011) similarly showed that CBF changes would be underestimated if the effect of
24 hyperoxygenation on blood T_1 was ignored and a further study showed no change in CBF
25 when correcting for the arterial blood T_1 decrease (Zaharchuk et al., 2008, Maleki et al.,
26 2011). The aCBV signal is derived solely from the arterial blood compartment due to the
27 suppression of the tissue signal by the rapid sequence of high flip angle pulses, and therefore
28 it is insensitive to any changes in tissue T_1 which in any case are likely to be small in the
29 brain. Although this simulation was carried out for aCBV weighted ASL data, these results
30 highlight the more general importance of accounting for any change in $T_{1\text{blood}}$ when fitting
31 ASL signal curves for both aCBV and CBF quantification.

32

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

1 al., 2009, Ito et al., 2003, Rostrup et al., 2000). The hyperoxia results presented here support
2 the findings of a recent ASL study (Mark et al., 2011) which found no significant change in
3 GM CBF in response to isocapnic hyperoxia. However, that ASL study did not correct for a
4 change in T_1 blood relaxation time which would have led to an underestimation of CBF,
5 potentially masking an actual increase CBF. It is likely that previously reported reductions in
6 CBF during hyperoxia measured with PC MRI can be attributed to the hypocapnia
7 accompanying the hyperoxia since CO_2 changes were not controlled (Rostrup et al., 1995,
8 Watson et al., 2000). It should be noted that the MBF and aCBV measures were from a
9 modest sample size in comparison to previous studies. However, previous reports of
10 vasoconstrictive effects of hyperoxia (Bulte et al., 2007b, Watson et al., 2000, Rostrup et al.,
11 2000, Rostrup et al., 1995) fall outside of the 95% confidence interval for our reported
12 measures of both MBF and aCBV, therefore it is likely that a change of such magnitude
13 would have been measured within this experimental design.

14

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

1

2 Alpha activity is often most prominent in the occipital lobe, where it is thought to reflect
3 activity of the thalamo-cortical loop; increasing in an eyes-closed wakeful resting state and
4 decreasing when the eyes are opened (Berger, 1929). The significant reduction in amplitude
5 of low frequency neural oscillations in the occipital lobe is consistent with the reduction in
6 alpha power in previous EEG recordings (Seo et al., 2007a). Traditionally, alpha activity was
7 thought to reflect cortical idling (Lopes da Silva et al., 1973, Berger, 1929, Niedermeyer and
8 Lopes da Silva, 1999), more recently, this has been updated to include the notion that
9 increased alpha activity represents increased functional inhibition (Jensen and Mazaheri,
10 2010, Jensen et al., 2012, Klimesch et al., 2007, Thut et al., 2006). The reduction in alpha in
11 response to hyperoxia in the occipital lobe may arise due to the greater sensitivity to alpha
12 power in this area, or reflect an increase in vigilance with supplementary O₂ (Moss et al.,
13 1998). Hyperoxia has been shown to enhance cognitive function to include long term
14 memory (Moss et al., 1998), working memory and word recall . The dynamic relationship
15 between altered vigilance, task performance and cerebral metabolism under hyperoxia needs
16 further investigation. Confounding effects due to patient discomfort and somatosensory
17 activation are reduced as it is difficult to perceive the difference between inspiring high levels
18 of oxygen required for hyperoxia and medical air, as highlighted by its use in placebo-
19 controlled trials (Ozkurt et al., 2012, Moss et al., 1998, Cohen et al., 2009).

20

21 These results have important implications for calibrated BOLD measurements using
22 hyperoxia (Chiarelli et al., 2007b, Goodwin et al., 2009, Driver et al., 2012), and the more
23 recent combined O₂ and CO₂ calibrated BOLD methods (Gauthier and Hoge, 2012, Bulte et
24 al., 2012). The results imply that hyperoxia based methods of BOLD calibration need not be
25 corrected for changes in baseline CBF or CBV (Chiarelli et al., 2007b) (hypercapnia based
26 BOLD calibration does not require any such correction since changes in CBF on hypercapnia
27 are implicit in the method). Hyperoxia can also be used to investigate vascular function and
28 structure, as highlighted by its recent use in venous CBV estimations (Blockley et al., 2012)
29 and vessel size imaging (Shen et al., 2011), techniques for which the lack of an effect of
30 hyperoxia on blood flow is important.

31

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

1 that are smaller in magnitude and spatial extent across subjects compared to iso-oxic
2 hypercapnia which implies that isocapnic hyperoxia is a more appropriate stimulus than
3 hypercapnia for calibrating the BOLD response (Chiarelli et al., 2007b). This said, the small
4 focal effect of hyperoxia on neural signals remains significant, suggesting that further
5 investigation is required in order to determine if the observed change may lead to an
6 under/overestimation of $CMRO_2$ in these areas. The significant but differential effects of
7 hyperoxia and hypercapnia on neural oscillatory processes may be of even greater importance
8 to the more recent combined calibrated BOLD models (Bulte et al., 2012, Gauthier and Hoge,
9 2012). Further investigation would allow quantification of separate hyperoxia and
10 hypercapnia $CMRO_2$ error terms in order to gain equivalent calibration constants which can
11 then be combined

12

13 **CONCLUSION**

14 This study addresses the main physiological assumptions made in hyperoxia-based calibrated
15 BOLD, and has found no significant effect of isocapnic hyperoxia on GM aCBV or global
16 MBF in the internal carotid arteries. While hyperoxia was shown to produce significant
17 changes in neuronal oscillations consistent with increased vigilance as measured using source
18 localised MEG, the changes were both more focal and of significantly smaller amplitude than
19 observed with hypercapnia (Hall et al., 2011). This supports the use of hyperoxia in
20 preference to hypercapnia for calibrating the BOLD response (Chiarelli et al., 2007b),
21 however further investigation is needed on the impact of changes in neuronal oscillations on
22 $CMRO_2$ consumption and the precision of combined hyperoxia-hypercapnia calibration.

23

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1 **TABLES:**

2

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

6

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

8

1 **Table 1**

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

2

1 **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, $T_{1\text{tissue}}$ (ms) ((Rooney et al., 2007)	2200
Arterial transit time, Δ_{arterial} (ms)	100
Bolus duration, W (ms)	1200
Equilibrium magnetisation, M_0	1
Simulated $T_{1\text{blood}}$ (ms) at 7T for FiO_2 (%) = 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

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1 **FIGURES**

2 Figure 1: Targeted respiratory paradigms for A) Experiment 1; isocapnic hyperoxia ($P_{ET}O_2 =$
3 500 mmHg) followed by 2 cycles of iso-oxic hypercapnia (subject specific baseline $P_{ET}CO_2 +$
4 8 mmHg) B) Experiment 2; isocapnic hyperoxia ($P_{ET}O_2 = 500$ mmHg), $P_{ET}CO_2$ targeted at
5 subject-specific baseline throughout and C) Experiment 3; 2 cycles of isocapnic hyperoxia
6 ($P_{ET}O_2 = 500$ mmHg), $P_{ET}CO_2$ targeted at subject-specific baseline throughout. Requested
7 end tidal pressure of O_2 is traced in black and CO_2 in grey.

8

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

18

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

23

24 Figure 4: A) Group maps of the significant reductions in neural oscillatory amplitude in
25 response to hyperoxia (3-15%), in the alpha (8-13Hz), beta (13-30 Hz) and gamma (30-50 Hz)
26 bands. B) This is contrasted with previously reported reductions from a hypercapnic stimulus
27 (3-15 %) (Hall et al. 2011) which produced a robust global desynchronisation, evident across
28 all subjects. C) The regions where a hypercapnia induced change is greater than a hyperoxia
29 induced change are shown in dark/light blue (0.5-7%). Regions where a hyperoxia induced
30 change are greater are shown in red/yellow (0.5-7%). In the hyperoxic case, changes are
31 smaller in magnitude and spatial extent, and are less robust across subjects. The reduction in
32 neural oscillatory power (mean \pm SEM) was interrogated across D)i) the motor cortex, ii) the
33 occipital lobe and iii) the medial frontal cortex in response to hyperoxia (black) and

- 1 hypercapnia (grey) (Hall et al. 2011). E)i-iii) This power change is further compared in the
- 2 same regions across the alpha, beta and gamma band.
- 3