Unexpected reductions in regional cerebral perfusion during prolonged hypoxia

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Cover Page

Title: Unexpected reductions in regional cerebral perfusion during prolonged hypoxia

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Running head: Hypoxia and regional cerebral blood flow

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Key Points Summary

- Cognitive performance is impaired by hypoxia despite global cerebral oxygen delivery and metabolism being maintained.
- Using arterial spin labeled (ASL) MRI, this is the first study to show regional reductions in cerebral blood flow (CBF) in response to decreased oxygen supply (hypoxia) at 2 h that increased in area and became more pronounced at 10 h.
- Reductions in CBF were seen in brain regions typically associated with the "default mode" or "task negative" network.
- Regional reductions in CBF, and associated vasoconstriction, within the default mode network in hypoxia is supported by increased vasodilation in these regions to a subsequent hypercapnic (5% CO_2) challenge.
- These results suggest an anatomical mechanism through which hypoxia may cause previously reported deficits in cognitive performance.

Abstract – 250-word max

Hypoxia causes an increase in global cerebral blood flow, which maintains global cerebral oxygen delivery and metabolism. Yet neurological deficits are abundant under hypoxic conditions. We investigated regional cerebral microvascular responses to acute (2 h) and prolonged (10 h) poikilocapnic normobaric hypoxia. We found that 2 h of hypoxia caused an expected increase in frontal cortical grey matter perfusion, but unexpected perfusion decreases in regions of the brain normally associated with the "default mode" or "task negative" network. After 10 h in hypoxia, decreased blood flow to the major nodes of the default mode network became more pronounced and wide spread. Use of a hypercapnic challenge (5% CO_2) confirmed that these reduction in CBF from hypoxia were related to vasoconstriction. Our findings demonstrate steady-state deactivation of the default network under acute hypoxia, which become more pronounced over time. Moreover, these data provide a unique insight into the nuanced localized cerebrovascular response to hypoxia not attainable through traditional methods. That reduced perfusion was observed in the posterior cingulate and cuneal cortex, regions thought to play a role in declarative

and procedural memory, provides an anatomical mechanism through which hypoxia may cause deficits in working memory.

Key words: Hypoxia, Cerebral Perfusion, ASL, DMN, Default Mode, CBF,

Abbreviations

ASL, arterial spin labeled; CBF, cerebral blood flow; DMN, default mode network; MNI Montreal Neurologic Institute;

Introduction

Despite the common use of the term "resting state," the human brain is never truly at rest. Demanding 15% of blood ejected from the heart and consuming almost 20% of total oxygen availability at rest, the brain is always metabolically active (Shulman *et al.*, 2004). Importantly, metabolic activity and thus oxygen consumption varies throughout the brain, which is paralleled by regional differences in blood flow and oxygen delivery, directly coupled to this metabolic activity (Fox *et al.*, 1988; Shulman *et al.*, 2004). Yet this extremely high metabolic rate renders the human brain exquisitely sensitive to hypoxia.

The classical understanding of cerebral physiology under acute moderate (altitudes, 1500-3500 m; SaO2, ~90%) and severe (altitudes, 3500 – 5500 m; SaO2, ~80%) hypoxia is that global cerebral blood flow (CBF) increases such that global oxygen delivery remains constant (Ainslie *et al.*, 2014) and global cerebral metabolic rate is unaffected (Kety *et al.*, 1948; Cohen *et al.*, 1967; Overgaard *et al.*, 2012; Willie *et al.*, 2015). Global cerebral metabolic rate is also thought to be maintained during prolonged moderate hypoxia (9 h, (Bailey *et al.*, 2009)) and with acclimatization to high altitude (Möller *et al.*, 2002). These data are however at odds with the suppression of cerebral metabolic activity seen in hypoxic tolerant animals (Hochachka, 1986; Hochachka *et al.*, 1996; Boutilier, 2001) and reported in altitude adapted humans (Hochachka *et al.*, 1994). Moreover, recently several studies have calculated cerebral metabolic rate non-invasively using magnetic resonance

imagining, whereby an unanticipated increase in cerebral metabolism was observed under poikilocapnic hypoxic conditions (Smith *et al.*, 2013; Xu *et al.*, 2012; Vestergaard *et al.*, 2016). Under such poikilopcapnic hypoxic conditions, hyperventilatory-induced hypocapnia will reduce cerebral tissue PCO_2 and extracellular pH with the potential to increase neuronal excitability via alterations in adenosine generation (Dulla *et al.*, 2005). Indeed, using MRI, a further report has shown that prophylactic low-dose acetazolamide, which may increase cerebral tissue PCO_2, abolishes the increase in cerebral metabolic rate of oxygen following six hours of normobaric poikilocapnic hypoxia (Wang *et al.*, 2015) Taken together, it is unclear exactly how cerebral metabolism is effected by acute or prolonged periods of poikilocapnic hypoxia.

Nevertheless, it is clear that hypoxia causes neurological deficits (Virues-Ortega *et al.*, 2004; Maiti *et al.*, 2008; Wagner, 2010) at altitude. Moreover, clinical conditions that involve periods of hypoxemia, such as sleep apnea, chronic obstructive pulmonary disease, anesthesia and stroke, all demonstrate concomitant cognitive declines. Thus the model of increased global CBF maintaining global metabolism in hypoxia may not reflect the complexity of regional cerebral metabolic and blood flow changes truly present. For example, brain regions with exceptionally high metabolism, such as the precuneus and the posterior cingulate, may be particularly vulnerable to reduced oxygen availability (DeVolder, 1990; Laureys *et al.*, 1999). Given this proposed regional susceptibility to hypoxia, regional measurements of CBF during prolonged periods of hypoxia under free-living conditions would be best suited to fully understand the brains true response. Indeed, the concept of regional differences in PO_2 across the brain despite maintained global cerebral oxygen delivery has been recently reviewed (see; Hoiland *et al.*, 2016; Ainslie *et al.*, 2016), adding further support that a finer grained examination of cerebral blood flow in hypoxia is needed. Unfortunately, to the best of the authors' knowledge, no such data exists for periods of hypoxia longer than 40 minutes (Buck *et al.*, 1998; Binks *et al.*, 2008; Pagani *et al.*, 2011; Micarelli *et al.*, 2013).

Consequently, the current study used arterial spin labeled (ASL) magnetic resonance imaging to quantify regional changes in cerebral microvascular perfusion. Images were

obtained in response to acute (2 h) and prolonged (10 h) exposure to poikilocapnic normobaric hypoxia (12% O_2). It was hypothesized that regional measures of CBF during hypoxia (particularly when prolonged) would allow determination of regions that showed increased CBF (most likely metabolically active areas of the brain e.g. cerebral cortex, grey matter) as well as regions that either did not show increases, or that may even show decreases. Determining blood flow responses to these later regions may shed light on the discrepancies between current reports of maintained global cerebral metabolism despite cognitive deficits that are suggestive of regional impairment.

Methods

This study and experimental design was approved by the ethics committee from the School of Sport Health and Exercise Sciences and the School of Psychology at Bangor University and Conformed to the Declaration of Helsinki (2008). Informed consent was obtained in writing after each participant was given a verbal and written explanation of the experimental protocol and fully understood the possible risks involved in taking part in the study. Procedures were carried out at the School of Sport, Health and Exercise Sciences (altitude = 20 m) or the Bangor Imaging Unit, in the School of Psychology (altitude = 50 m).

Participants

Thirteen males (age = 26 (sd 6) yrs; body mass = 77 (sd 12) kg; height = 182 (sd 9) cm) volunteered to take part. Exclusion criteria were any clinically diagnosed primary headache disorder and ascent to altitude above 1500 m in the previous 6 months.

Experimental Design

The experimental design has been reported in full in three other papers (Lawley *et al.*, 2013; 2014*a*; 2014*b*) which investigated other imaging and physiologic endpoints: water mobility and diffusion changes, and brain volumetric and intracranial pressure changes. The ASL data, presented for the first time in this paper, was collected at the same time as the other imaging endpoints but has not been reported except in abstract form. Participants reported to the laboratory at 07:00 h on two separate occasions separated by

at least 5 days having been asked to refrain from alcohol consumption and exhaustive exercise for a period of 48 h before each visit. To maintain adequate hydration participants were instructed to drink 40 ml/kg of water in the 24 h prior to each visit, with an *ad libitum* diet, which was recorded and repeated in both trials. The controlled fluid and nutritional intake minimized the possibility of systemic dehydration effecting central fluid dynamics.

Trials consisted of either a 10 h normobaric normoxia (21% O_2) or normobaric hypoxia (12% O_2) in a temperature (23°C) and humidity (40%) controlled environmental chamber. Participants were blinded to the experimental conditions and assigned to each exposure in a randomized order (http://www.randomization.com). Magnetic resonance imaging was performed after 2 and 10 h exposure in both trials. Importantly, the same oxic state (21% O_2 or 12% O_2) was maintained during transportation to and throughout the magnetic resonance imaging, through the use of appropriate gaseous mixtures through a facial mask fitted with a one-way valve to minimize rebreathing of expired respiratory gases. Magnetic resonance imaging sequences were obtained after 10-min supine rest. During the MRI acquisition, a short (5 min) hypercapnic (5% carbon dioxide) challenge was also introduced. Hypercapnia elevates the partial pressure of CO_2 in arterial blood and is a potent cerebral vasodilator. Thus, comparing CBF before and during the hypercapnic challenge gave an indication of the cerebral microvascular capacity to dilate.

Cardiorespiratory variables

Oxygen saturation and heart rate were monitored continuously in the environmental chamber (A&D Medical, TM-2564GP, CA, USA). Use of the MRI system's in vivo monitoring equipment allowed pulse oximetry, heart rate and vector cardio-grams to the obtained (Phillips Medical, Eindhoven, NL). Haemoglobin was sampled from the ear-lobe capillary at the end of each visit (HemoCue Photometer, Sheffield) and arterial oxygen content was calculated as (haemoglobin x 1.39 x (oxygen saturation/100), which excludes oxygen dissolved in plasma). During the final minute of each MRI measurement, end tidal carbon dioxide was sampled and analysed by fast responding gas analyser (Servomex, I.R. gas analyser, PA404, Sussex, UK).

Magnetic Resonance Imaging Acquisition and Post-processing

All magnetic resonance imaging sequences were conducted on a 3 Tesla magnetic resonance imaging scanner (Phillips Achieva, Philips Healthcare, Eindhoven, Netherlands) using a 16 channel head and neck coil. All imaging sequences were acquired with sensitivity encoding for fast magnetic resonance imaging (SENSE).

Images

Acquisition: A high resolution T_1 weighted image was acquired and used for registration of ASL data to a standard template. The T_1 weighted image was acquired as 5 echo MP-RAGE sequence (TE = 3.5, 5.1, 6.8, 8.5, 10.2 ms, TR = 12 ms, TI = 1150 ms, 3D acquisition, FOV = 240 mm X 220 mm X 130 mm, voxel dimensions = 0.7 X 0.7 X 0.7 mm^3).

Single-phase arterial spin labelled images were acquired using the Philips clinical science provided ASL package. This is based on an echo planar acquisition using the STAR ASL labeling. Labelling of inflowing blood was achieved through a parallel slab applied 20 mm below the acquisition slices (slab thickness 100 mm, delay 1600 ms). ASL is limited in the number of slices, and therefore coverage, that can be obtained in a single scan. Typically, inflowing blood is tagged using an RF pulse, then after a short delay images are taken of a region into which the tagged blood will have traveled during the delay. Imaging too many slices will lead to a situation where inflowing blood will have either left the lower regions, or not reached the upper regions, in the interval between the RF tag and image acquisition. As such, we were only able to cover a portion of the brain in this experiment. Specifically, 12 slices at 2mm X 2mm in plane resolution with 256mm X 256mm FOV and 6 mm slice thickness were acquired aligned to the AC-PC axis, with the bottom most slice covering the corpus callosum, providing coverage of the top half of the brain. Slices were acquired as 40 tagged and control pairs with TR of 3 secs, and a TE of 15 ms, giving a total scan time of 4 mins. ASL data was acquired during baseline normoxia/hypoxia and during a short (5 min) hypercapnic (5% CO_2 added to inspired gas) challenge. The increase in blood flow that occurs with breathing CO_2 enabled vascular reactivity to be assessed.

Analysis: Imaging data where exported from the scanner as dicom images before being converted to NIFTI format (dcm2nii, http://www.mccauslandcenter.sc.edu/mricro/mricron/dcm2nii.html). All image analysis was then run using the tools provided in the FMRIB Software Library (FSL) v5.0. T_1 weighted images were first brain extracted using FSL's BET option (Smith, 2002) then segmented using FAST (Zhang *et al.*, 2001). ASL data was analyzed using BASIL (Chappell *et al.*, 2009), assuming a blood T_1 of 1.66s in normoxia (Lu *et al.*, 2004) and a change to 1.61s in hypoxia (Lu *et al.*, 2004; Harris *et al.*, 2013) due to the reduced O_2 saturation. The cerebral blood flow (CBF) maps produced by BASIL were then registered to the T_1 weighted structural images and smoothed with a Gaussian 4 mm kernel before being masked with the grey matter image from the T_1 segmentation. The T_1 weighted images where then registered to the Montreal Neurological Institute (MNI) T_1 weighted average image supplied with FSL using the FLIRT toolbox, and this registration applied to the grey matter masked CBF images. The grey matter CBF images were then compared using RANDOMISE (Winkler *et al.*, 2014) with cluster based thresholding for correction of multiple comparisons using cluster mass at a $p< 0.01$. MRI scanning was performed in normoxia and hypoxia at 2 and 10 h giving four time points – normoxia-morning, normoxia-afternoon, hypoxia-morning and hypoxia-afternoon. Statistical comparisons were completed between conditions (normoxia vs. hypoxia) at 2 h and then again at 10 h, as well as within conditions (2 h vs. 10 h for normoxia and hypoxia). Images shown represent the change in blood flow between hypoxia and the respective normoxic time points thus controlling for potential diurnal variations.

Vascular dilation to the hypercapnic challenge was assessed by collecting ASL data as above. Hypercapnia was induced by adding 5% CO_2 to the atmosphere that participants were breathing (normoxic or hypoxic atmosphere). Subtracting the CBF maps for normal breathing from the hypercapnic breathing generated an image showing regions that exhibited increases in CBF, corresponding to vascular dilation. A one sample T-Test was then run in RANDOMIZE to compare normoxia to hypoxia for the 2 and 10 h time points with cluster based thresholding for correction of multiple comparisons using cluster mass at a $p< 0.01$ (figure 3).

Results

Acute cerebral blood flow responses to hypoxia.

To determine the effect of hypoxia on regional CBF, human subjects were exposed to poikilocapnic normobaric hypoxia (12% O_2) for 10 h, which caused significant haemoglobin desaturation (~81 %), reduced arterial oxygen content (Δ-3 $mL \cdot dL^{-1}$) and hypocapnia (Δ-5 mmHg). We have previously reported that this protocol causes significant acute (2 h) increases in global CBF measured at the level of the external carotid and vertebral arteries (Lawley *et al.*, 2014*a*), such that global cerebral oxygen delivery remains normal. Resting steady-state regional CBF was stable over the course of a day in normoxia, with no significant differences between 2 and 10 h.

As expected, regional CBF increased after 2 h in hypoxia, with significant clusters (cluster based correction $p<0.01$) in the frontal pole, middle frontal gyrus, anterior division of the cingulate gyrus, the superior frontal gyrus, and the precentral gyrus, amongst others. Unexpectedly, small regional decreases in blood flow were also observed after 2 h of hypoxia, predominantly in the posterior cingulate gyrus, but also in the cuneal cortex and the supramarginal gyrus (cluster based corrections at $p<0.01$), all regions normally associated with the Default Mode Network (DMN) and sensitive to neural degeneration (Raichle *et al.*, 2001; Buckner *et al.*, 2005; Cole *et al.*, 2014). Figure 1 shows regions that exhibited increases (red) and decreases (blue) in CBF (uncorrected voxel-wise $p < 0.05$, to improve display of the regions exhibiting changes in CBF) after 2 h of hypoxia. MNI Co-ordinates for the top 11 significant clusters are provided in tables 1 and 2.

Cerebral blood flow response to prolonged poikilocapnic hypoxia.

Physiological adaptations to hypoxia are dynamic and tend to develop over time (Ainslie & Subudhi, 2014). For example, ventilation progressively increases over time, causing progressive hypocapnia (Smith *et al.*, 2001). Thus, measuring regional CBF after 10 h of hypoxia provided the opportunity to assess the stability of these responses. After ten hours in hypoxia, cerebral regions that previously showed an increase in flow (after 2 h in hypoxia) had returned towards normoxic levels (10 h normoxia vs 10 h hypoxia

comparison). In contrast, the decreased cerebral blood flow that occurred within the DMN after 2 h in hypoxia was exacerbated after 10 h in hypoxia (10 h normoxia vs 10 h hypoxia comparison). Again decreases in CBF were predominantly in the posterior cingulate, but also extended to the superior division of the lateral occipital cortex, aspects of the anterior cingulate gyrus, the frontal pole, postcentral gyrus and the supramarginal gyrus, amongst others (cluster based corrections at $p<0.01$). Figure 2 shows regions that exhibited decreases in CBF (blue) after 10 h of hypoxia (uncorrected voxel-wise $p < 0.01$ to improve display of the regions exhibiting decreased CBF). Individual CBF in absolute units of ml.100 $g^{-1}.min^{-1}$ for those regions that showed a statistically significant difference between hypoxia and normoxia are also presented in figures 1 and 2 for both 2 and 10 h. MNI co-ordinates for the top 11 significant clusters are provided in table 3.

Increased vascular tone in the default network during hypoxia revealed by a vasodilatory challenge.

To confirm the presence of localized regions of vasoconstriction with the DMN, we exposed participants to 5% CO_2 after 2 h and 10 h in hypoxia. The hypercapnic challenge presented after acute hypoxia resulted in normalisation of oxygen saturation (SaO2 = 96%) with increases in CBF at 2 h within posterior components of the DMN (that had previously showed reduced perfusion after 2 h in hypoxia (figure 3)). Consistent with the increase in regions showing reduced CBF from 2 h to 10 h during steady state, regions of vasodilatation in response to the CO_2 challenge increased after 10 h of hypoxia. Figure 3 shows areas of dilation in response to hypercapnia after 2 h (blue) and 10 h (red) of exposure to hypoxia (cluster based correction at $p< 0.01$). Note these vascular haemodynamics overlap with the steady-state CBF decreases seen in figures 1 and 2. Also note when comparing the vascular changes in response to a CO_2 challenge in hypoxia with normoxia, we see that the increase in CBF was greater during hypoxia at the 2 h time point (figure 3, yellow region, cluster corrected $p < 0.05$), but not at 10 h.

Discussion

Overview main findings.

The classical understanding of cerebral physiology suggests that exposure to hypoxia causes both hypoxia-induced vasodilation and hypocapnia-induced vasoconstriction (Ainslie & Subudhi, 2014). An increase in global CBF suggests a dominance of vasodilation. The present study extends these classical findings by using advanced MRI techniques to quantify regional CBF. The present study demonstrated that acute (2 h) and prolonged (10 h) hypoxic exposure led to heterogeneous changes in resting cerebral microvascular perfusion. In particular, the results demonstrate profound and unexpected reductions in CBF within regions of the DMN at 2 h, which were exacerbated at 10 h. Further support for this finding of vasoconstriction within the DMN was obtained by CO_2 inhalation. This experimental manipulation would predictably dilate constricted blood vessels and indeed resulted in greater vasodilation across brain regions that almost perfectly overlapped the hypoxia induced vasoconstriction within the DMN.

Previous data on cerebral blood flow and hypoxia

Changes in cerebral perfusion under hypoxic conditions are extremely dynamic. Acutely, CBF increases (Binks *et al.*, 2008; Imray *et al.*, 2014; Lewis *et al.*, 2014; Lawley *et al.*, 2014*a*) especially if $PaCO_2$ is maintained via end-tidal clamping procedures (Willie *et al.*, 2012). However, regional changes in cerebral blood flow are heterogeneous acutely (Binks *et al.*, 2008; Pagani et al., 2000; Buck et al., 1998; Pagani et al., 2011; Micarelli et al., 2013). Over the ensuing hours of poikilocapnic hypoxia (~6 h, (Imray *et al.*, 2014; Lewis *et al.*, 2014)), global cerebral perfusion begins to fall and even returns towards normoxic values after 10 h (Lawley *et al.*, 2014*a*) when measured at the level of the extra-cranial arteries (Lawley *et al.*, 2014*a*). The current data obtained using an independent technique further suggests a decrease in blood flow in the cerebral capillaries. Under these or similar (12.9% O_2, (Bailey *et al.*, 2011)) conditions, cerebral oxygen delivery is reduced. Given the tight metabolic-flow coupling across the brain (Raichle *et al.*, 2001), our findings of reduced localized perfusion strongly suggest the human brain down-regulates regional energy turnover, specifically within areas of DMN that characteristically display high metabolic rates.

Mechanisms for decreased regional cerebral blood flow in hypoxia

Metabolism. Regional CBF parallels local neural activity (Raichle *et al.*, 2001). Indeed, it is well established from the neuroimaging literature that increased neural activity leads to elevated blood flow to meet the associated metabolic demands (Attwell & Iadecola, 2002; Shulman *et al.*, 2004; Peppiatt & Attwell, 2004; Aubert *et al.*, 2007). Conversely, decreases in neural activity produce concomitant reductions in CBF (Shmuel *et al.*, 2002; Pasley *et al.*, 2007). This suggests an intriguing proposition that regional decreases in CBF in response to hypoxia are due to de-activation (reduced metabolic activity) of those regions. That these reductions are found in the DMN further supports this proposition.

In the resting state, a default mode of increased brain function, neural activity and blood flow is seen within the medial prefrontal cortex, posterior cingulate cortex, retrosplenial cortex and inferior parietal lobe (the DMN). This has led to the suggestion that the increased activity of these regions at rest facilitates gathering of information from the external and/or internal environment (Raichle *et al.*, 2001; Gusnard & Raichle, 2001) in a non-directed fashion. When the brain is no longer at "rest "and engaged in a task, activity in these regions is attenuated (Raichle *et al.*, 2001; Buckner & Vincent, 2007; Raichle & Snyder, 2007). It is generally accepted that deactivation of the DMN during a given task reduces total metabolic load and mobilizes resources to support task associated behaviors. In traditional task-based fMRI studies, this is thought to be due to an acute increase in the energy requirements of brain regions involved in the task. In our study however, participants were not engaged in a task and were lying quietly inside the MR scanner under steady-state conditions. We therefore suggest that the steady-state deactivation of the DMN we report, as reflected by reduced CBF, is likely due to maintenance of homeostatic regulation (oxygen supply-demand).

The homeostatic regulation interpretation is somewhat consistent with Hochachka et al.'s classic model of hypoxia tolerance (Hochachka *et al.*, 1996) and although speculative, in line with measured reductions in glucose metabolism in lowlanders acclimatized to high altitude (Hochachka *et al.*, 1999) and Quechua high altitude natives (Hochachka *et al.*, 1994). However this finding is in contrast to the extensive literature reporting unchanged global cerebral metabolism (measured directly by arterial-jugular cannulation) in humans

under most hypoxic conditions (Cohen et al. 1967; Kety & Schmidt 1948; Overgaad et al. 2012; Ainslie et al. 2013, Moller et al., 2002, Willie et al., 2015). At present the cause of this discrepancy is unclear, but at least three explanations are conceivable. First, global arterial-venous blood sampling across the brain does not possess the precision to detect subtle microvascular alterations as observed in the current study. Second, cerebral metabolism has predominantly been measured acutely (within minutes) or after successful acclimatization (days at altitude) when physiological adaptations, such as increased cerebral blood flow or haemoglobin concentration, maintain oxygen delivery to the brain (Kety *et al.*, 1948; Cohen *et al.*, 1967; Möller *et al.*, 2002; Overgaard *et al.*, 2012; Ainslie *et al.*, 2014). Third, if cerebral metabolism is normal, our observations suggest regional reductions in CBF, possibly concomitant with increased cerebral arterial blood volume, could be a mechanism through which the brain defends against hypoxia by increasing mean capillary transit time, perfusion heterogeneity and facilitating oxygen and glucose extraction (Jespersen & Ostergaard, 2012). A similar counterintuitive reduction in sublingual microcirculatory blood flow has been observed (Martin *et al.*, 2010). The only study to measure global cerebral metabolism in healthy subjects using a similar protocol to that herein (9 h at 13 % O_2) noted increased cerebral oxygen extraction in the face of reduced oxygen delivery (Bailey *et al.*, 2009). According to Raichle et al. (2001), such observations would actually suggest a decrease in global neural activity, due to the tight coupling between CBF and oxygen consumption at rest.

Hypocapnia. Under free breathing conditions, hypoxia causes hypocapnia and respiratory alkalosis (Dempsey & Forster, 1982; Duong, 2007), which together are powerful cerebral vasoconstrictors and modulators of cerebral metabolism. Regional differences in cerebral vasoconstrictor response to acute hypocapnia have previously been observed (Ito *et al.*, 2000; Schlunzen *et al.*, 2010), but importantly without clear uniformity to the DMN. Moreover, while pH is a potential modulator of cerebral metabolism, the observed hypocapnia and resultant alkalosis should theoretically *increase* cerebral metabolism (Chen & Pike, 2010; Jain *et al.*, 2011), which is in contrast to the observed reductions in cerebral metabolism as inferred from reductions in cerebral perfusion as observed herein. It is also worth noting that the impact of pH on cerebral metabolism is not without

controversy, as reductions in global cerebral metabolism have been noted with mild hypocapnia (Xu *et al.*, 2011; Kliefoth *et al.*, 1979). In the current study, the magnitude of hypocapnia was mild, whereas recent data suggest that severe changes in cerebral pH are required to alter cerebral metabolism (Bain *et al.*, 2016), at least as it pertains to hypercapnic hypoxia in elite apnea divers. Taken together it seems that mild hypocapnia may be responsible for the global fall in CBF between 2 and 10 h in hypoxia, but at present is unlikely to explain regional hypoperfusion within the DMN.

Altitude symptoms. Prolonged hypoxia caused symptoms of altitude illness (headache, nausea, fatigue, etc.), Lake Louise Score, 4(3) at 10 h in hypoxia) in the majority of this cohort, which we have reported previously (Lawley *et al.*, 2014*b*). Allocation of neural resources to the perception of pain and discomfort may have interrupted freethinking and caused deactivation of the DMN. However, very few individuals reported symptoms after 2 h in hypoxia despite clear evidence of regional hypoperfusion in the DMN. Moreover, no relationships were observed between symptoms of altitude illness at 10 h in hypoxia and regional reductions in cerebral blood flow. Collectively these data suggest that there is no link between symptoms of altitude illness and decreases in cerebral blood flow within the DMN in hypoxia.

Implications of reduced default mode network perfusion

Cognition at altitude. Neurocognitive performance is substantially reduced with severe hypoxia (Subudhi *et al.*, 2014) including memory functions (Huppert, 1982; Hornbein, 2001; Banderet & Shukitt-Hale, 2002; Maiti *et al.*, 2008; Wagner, 2010). But preservation of global cerebral oxygen delivery and metabolism acutely (Kety *et al.*, 1948; Cohen *et al.*, 1967; Möller *et al.*, 2002; Overgaard *et al.*, 2012; Ainslie *et al.*, 2014; Willie *et al.*, 2015) and with acclimatization to hypoxia made these observations difficult to explain (Möller *et al.*, 2002). The posterior cingulate and cuneal cortex are thought to play a role in declarative and procedural memory (Laureys *et al.*, 1999), hence the observed reductions in activity within these regions could conceivably explain decrements in memory function with hypoxia. However we recognize this can only be speculated from our data as we did not

collect measures of cognitive function.

Hypoxia as a unifying theory of default network deactivation in cerebral disease.

Normally, reduced activity in the DMN is associated with reductions in CBF. This is non-deleterious because blood that does arrive is sufficiently oxygenated to provide a "luxury perfusion effect" for basal metabolism. However, in conditions of environmental hypoxia (as herein) and in cerebral disease this may not be the case. Reductions in default network perfusion and/or metabolism have been observed in sleep apnea (Binks *et al.*, 2008; Shiota *et al.*, 2013; Santarnecchi *et al.*, 2013; Buratti *et al.*, 2014; Osorio *et al.*, 2014) and neurocognitive dysfunction is a hallmark feature of sleep apnea (Findley *et al.*, 1986; Gagnon *et al.*, 2014; Vaessen *et al.*, 2015), whilst treatment of nocturnal hypoxia in sleep apnea patients improves DMN functional connectivity and task-related deactivation of the DMN (Cooke *et al.*, 2009; Prilipko *et al.*, 2014; Troussière *et al.*, 2014), and enhances memory (Dalmases *et al.*, 2015). A similar regional cerebral hypoperfusion (Smith *et al.*, 1999; Nicolakakis & Hamel, 2011) and parenchyma hypoxia (Zlokovic, 2011) has also been observed in Alzheimer's disease that is associated with cognitive impairment and disease progression. These clinical observations, alongside the current findings, make a compelling (albeit indirect and speculative) case for future research to examine the hypothesis that intermittent and/or continuous hypoxia may lead to chronic deactivation of the DMN.

Limitations

A limitation of the current study is the lack of a concomitant assessment of neurocognition. Thus, any link between the observed reductions in cerebral blood flow in hypoxia and neurological deficits are highly speculative; yet anatomical parallels between brain regions with reduced blood flow and those known to be involved in memory is supportive of this proposition. Another minor limitation was our inability to control PaO_2 during 5% carbon dioxide breathing inside the MRI scanner. Breathing carbon dioxide increases ventilation and normalizes oxygen saturation. Thus, the effect of hypoxia on the cerebral vasculature may have been removed, making it unclear as to which stimulus or combination thereof (increased PaO_2 - removal of hypoxia, or increased $PaCO_2$ - hypercapnia) may be driving the

measured changes in CBF. For clarity, the interpretation of a greater relative vasodilation in the DMN during CO_2 breathing in hypoxia could be confounded by the removal of hypoxic vasodilation in other brain regions. However, after 10 h in hypoxia, there was no statistical evidence of vasodilation and yet CO_2 breathing still caused increased vasodilation in the DMN. Thus, overall, our interpretation is the same, that prolonged hypoxia leads to localized vasoconstriction, and reduced CBF within the DMN. Admittedly it is clear if the hypercapnic challenge eliminated hypoxic dependent effects by increasing ventilation and SaO2, or caused a greater CO_2 induced vasodilation (or a combination thereof). Again, this does not change the fact that vasodilation occurred, nor refute our primary finding of reduced CBF in hypoxia within these regions. To further aid this conclusion, comparisons between the hypercapnic response in normoxia with that in hypoxia, showed a greater increase in CBF during hypoxia in these regions(figure 3). Thus it is unlikely that the greater vasodilation in the DMN in hypoxia is explained by inherently greater reactivity to CO_2.

Summary

Our results show that in hypoxia the human cerebral circulation alters regional perfusion, especially in areas that normally have high metabolic activity at rest. This suggests that the reduction of CBF reported herein is an attempt to adapt to metabolic demand in other regions of the brain. Usually this demand is created by activation of other brain regions, but in hypoxia is created by the low oxygen environment. These results therefore provide exciting evidence of the microcirculatory effects of metabolic challenges and localized cerebral hypoxia in the normally healthy population, and suggest novel avenues of investigation in disease states with a hypoxic component.

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Additional Information

Competing interests

The Authors hold no competing interests with regards to the content of this manuscript.

Author Contributions

All experimental procedures were carried out at Bangor University. Hypoxic exposure was carried out in the Extremes Research Group laboratory in the School for Sports Health and Exercise Sciences, while imaging was performed in the Bangor Imaging Unit within the School of Psychology.

The experiment was conceptualised and designed by JSL, SJO, JHM and PGM. Data

acquisition was performed by JSL and PGM. Analysis of the arterial spin labelled images was performed by PGM. All authors were involved in the interpretation of final results and writing of the manuscript.

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Authors Translational perspective

Hypoxia causes decrements in cognitive performance, slowing simple and complex reaction times and impairing memory. To our knowledge, this is the first study to show that when oxygen is in short supply, the human brain responds by reducing blood flow to specific portions of the brain that typically consume lots of oxygen. Assuming there exists a consistency of metabolic-flow coupling in hypoxia as in normoxia, these data document that, like many hypoxic tolerant animals, the human brain is capable of suppressing local neural activity in a bid to reduce overall metabolic demand. That reduced perfusion is observed in brain regions that govern memory and other cognitive processes suggests an anatomical mechanism through which hypoxia may cause deficits in cognitive performance.

Tables

Table 1 – MNI co-ordinates for 11 largest clusters showing increases in CBF after 2 h of hypoxia (cluster-wise correction p < 0.01)

Cluster Index	Num. of Voxels	MAX	MAX X (mm)	MAX Y (mm)	MAX Z (mm)	COG X (mm)	COG Y (mm)	COG Z (mm)
11	149	0.828	36	54	26	37.9	39.8	38.1
10	135	0.826	-18	62	24	-18.2	54.4	34.8
9	112	0.782	-4	-18	38	-0.304	-18.8	43.7
8	95	0.743	32	-4	48	37.3	-1.07	58.8
7	79	0.72	-56	-10	44	-44.9	-17.1	61.2
6	76	0.697	64	-22	40	57.6	-33.6	51.4
5	75	0.708	2	54	30	9.17	57.5	35.7
4	54	0.613	-30	0	60	-30.5	3.41	65.1
3	38	0.528	44	6	36	46.5	11.8	42
2	32	0.479	44	-58	56	42.2	-52.1	59.8
1	31	0.45	20	38	46	14	43.4	49.2

Table 2 - MNI co-ordinates for 11 largest clusters showing decreases in CBF after 2 h of hypoxia (cluster-wise correction p < 0.01)

Cluster Index	Num. of Voxels	MAX	MAX X (mm)	MAX Y (mm)	MAX Z (mm)	COG X (mm)	COG Y (mm)	COG Z (mm)
11	183	0.865	2	-44	24	-0.481	-42.5	32
10	56	0.645	20	-74	24	16.7	-72.4	28.6
9	27	0.449	-66	-28	24	-64.4	-31	29.2
8	21	0.383	-44	-64	38	-46.3	-62.6	39
7	19	0.337	-24	36	24	-27.7	35.9	27.7
6	17	0.314	58	-32	30	54	-31.1	32
5	12	0.217	-42	-38	38	-35.8	-40.3	41.3
4	11	0.189	-10	-78	24	-13.1	-75.6	24.2
3	10	0.177	-62	-46	24	-64.6	-44	25.4
2	9	0.142	-26	12	46	-26	13.3	48.2
1	8	0.152	-42	-68	24	-42.8	-67.5	25.8

Table 3 - MNI co-ordinates for 11 largest clusters showing decreases in CBF after 10 h of hypoxia (cluster-wise correction p < 0.01)

Cluster Index	Voxels	MAX	MAX X (mm)	MAX Y (mm)	MAX Z (mm)	COG X (mm)	COG Y (mm)	COG Z (mm)
11	1485	0.968	-16	-78	24	0.935	-54.5	34.3
10	599	0.928	4	28	24	-16.1	39.5	36.1
9	332	0.876	62	-6	24	50	13.5	31.6
8	271	0.85	50	34	24	30.8	48.8	28.4
7	174	0.792	60	-34	24	62.3	-30.9	31.2
6	129	0.754	-60	-52	24	-61.2	-40.4	28.4
5	111	0.726	34	-50	36	39.5	-52.7	45.8
4	96	0.702	62	-48	24	56.3	-47.7	31.9
3	74	0.636	32	32	30	32.4	34.5	36.8
2	55	0.576	30	-74	36	33.6	-70.8	40.4
1	45	0.536	-42	-60	24	-45.8	-62.5	35.7

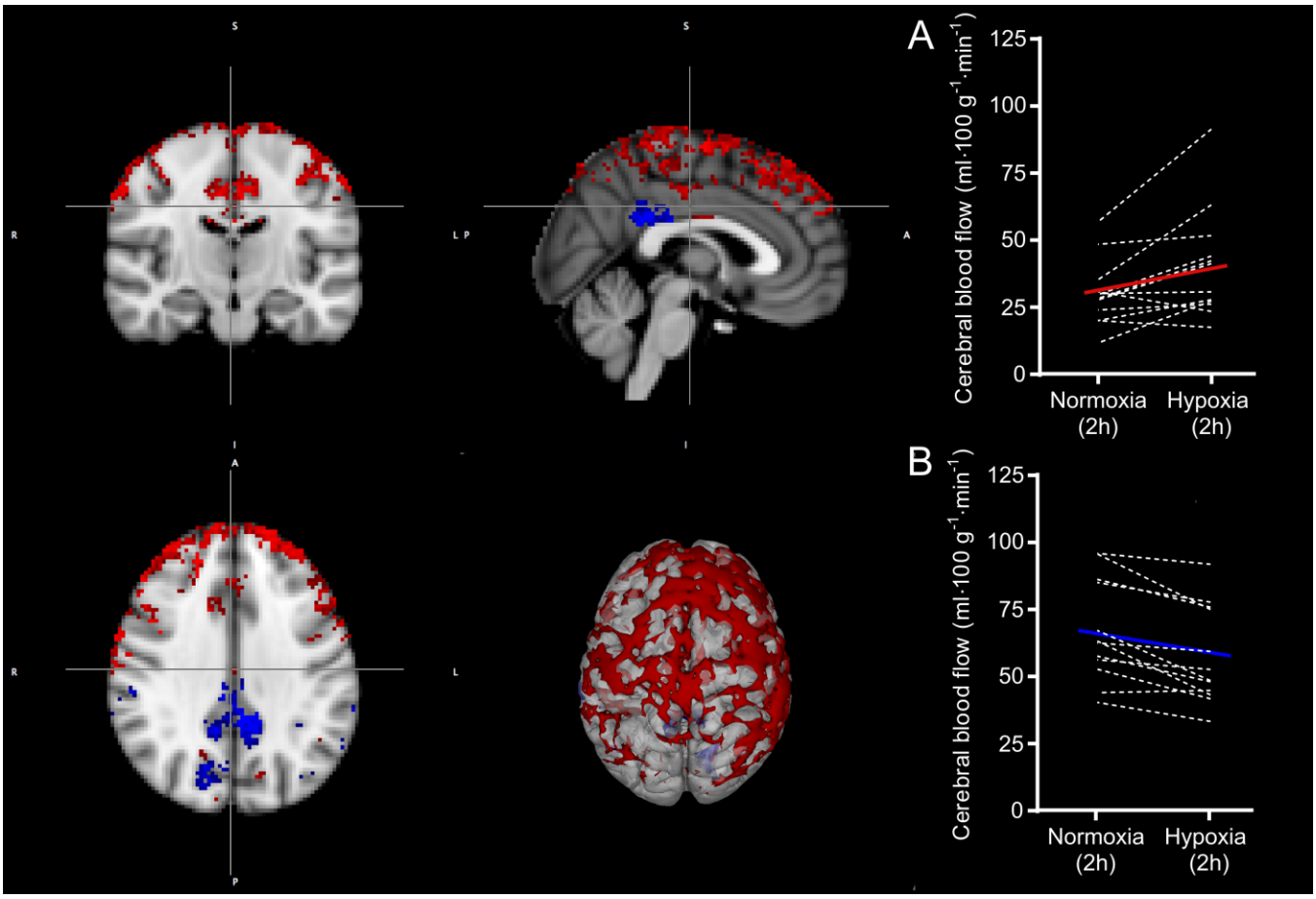
Figure legends

Figure 1. After 2 h of hypoxia regional CBF is seen to vary when compared to normoxia with increases (red) and decreases (blue) in CBF seen. Significant clusters (cluster based correction $p<0.01$) in the frontal pole, middle frontal gyrus, anterior division of the cingulate gyrus, the superior frontal gyrus, and the precentral gyrus, amongst others, showed expected increases in CBF (absolute CBF values in A), while unexpected decreases in regional CBF (absolute CBF values in B) were seen predominantly in the posterior cingulate gyrus, but also in the cuneal cortex and the supramarginal gyrus (uncorrected voxel-wise $p < 0.05$, to improve display of the regions exhibiting changes in CBF).

Figure 2. Regional variations in CBF after 10 h of hypoxia saw a normalization of blood flow in cortical areas that had shown an increase at 2 h. However regions of the DMN that had shown decreased CBF at 2 h, increased in extent at 10 h. Decreases in CBF were found predominantly in the posterior cingulate, but also extended to the superior division of the lateral occipital cortex, aspects of the anterior cingulate gyrus, the frontal pole, postcentral gyrus and the supramarginal gyrus, amongst others (cluster based corrections at $p<0.01$). Regions that exhibited decreases in CBF are shown in blue (uncorrected voxel-wise $p < 0.01$ to improve display) with absolute changes shown in (A).

Figure 3. Areas of vascular dilation in response to hypercapnia are after 2 h (blue) and 10 h (red) of exposure to hypoxia (cluster based correction at $p< 0.01$). The hypercapnic challenge presented after acute hypoxia resulted in increases in CBF at 2 h within posterior components of the DMN (that had previously showed reduced perfusion after 2 h in hypoxia). Consistent with the increase in regions showing reduced CBF from 2 h to 10 h during steady state, regions of vasodilatation in response to the CO_2 challenge increased after 10 h of hypoxia. Note these vascular haemodynamics overlap with the steady-state CBF decreases seen in figures 1 and 2.

Figures



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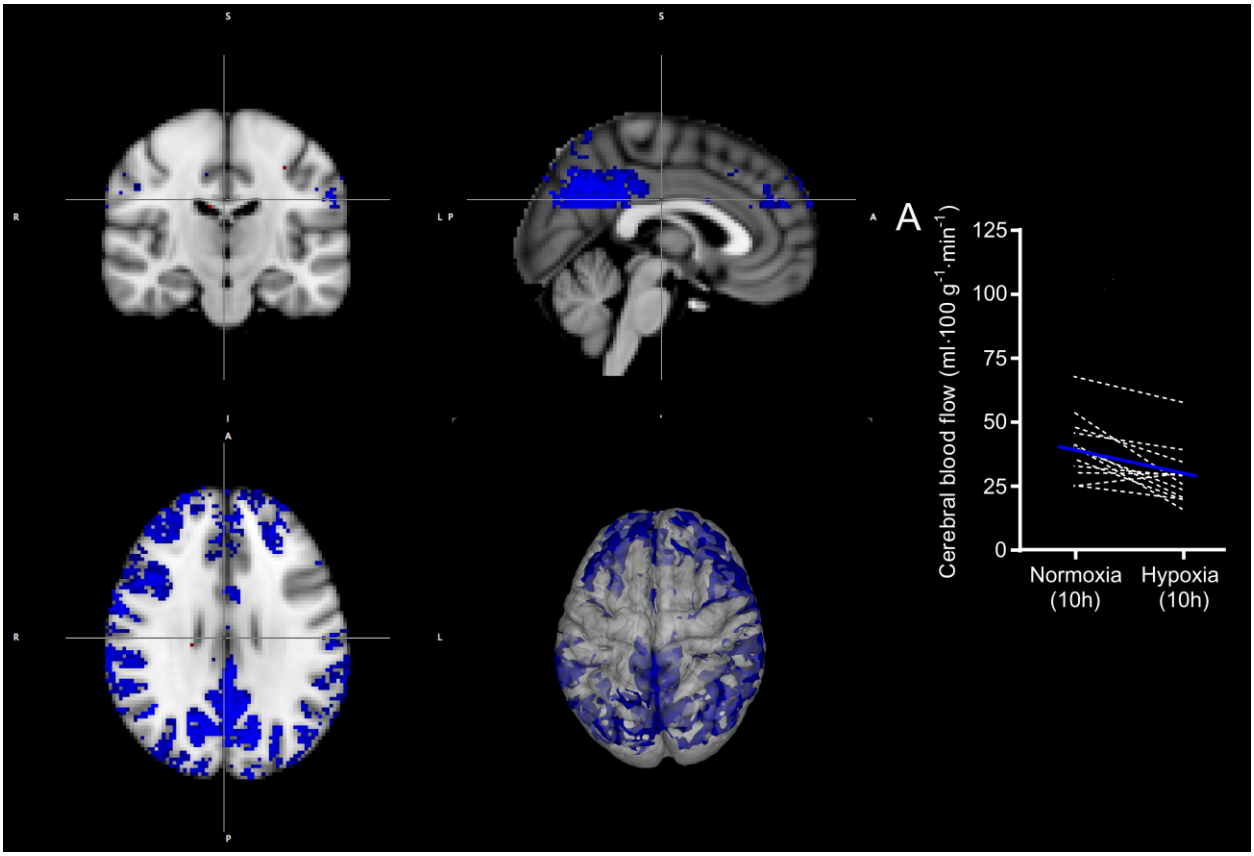

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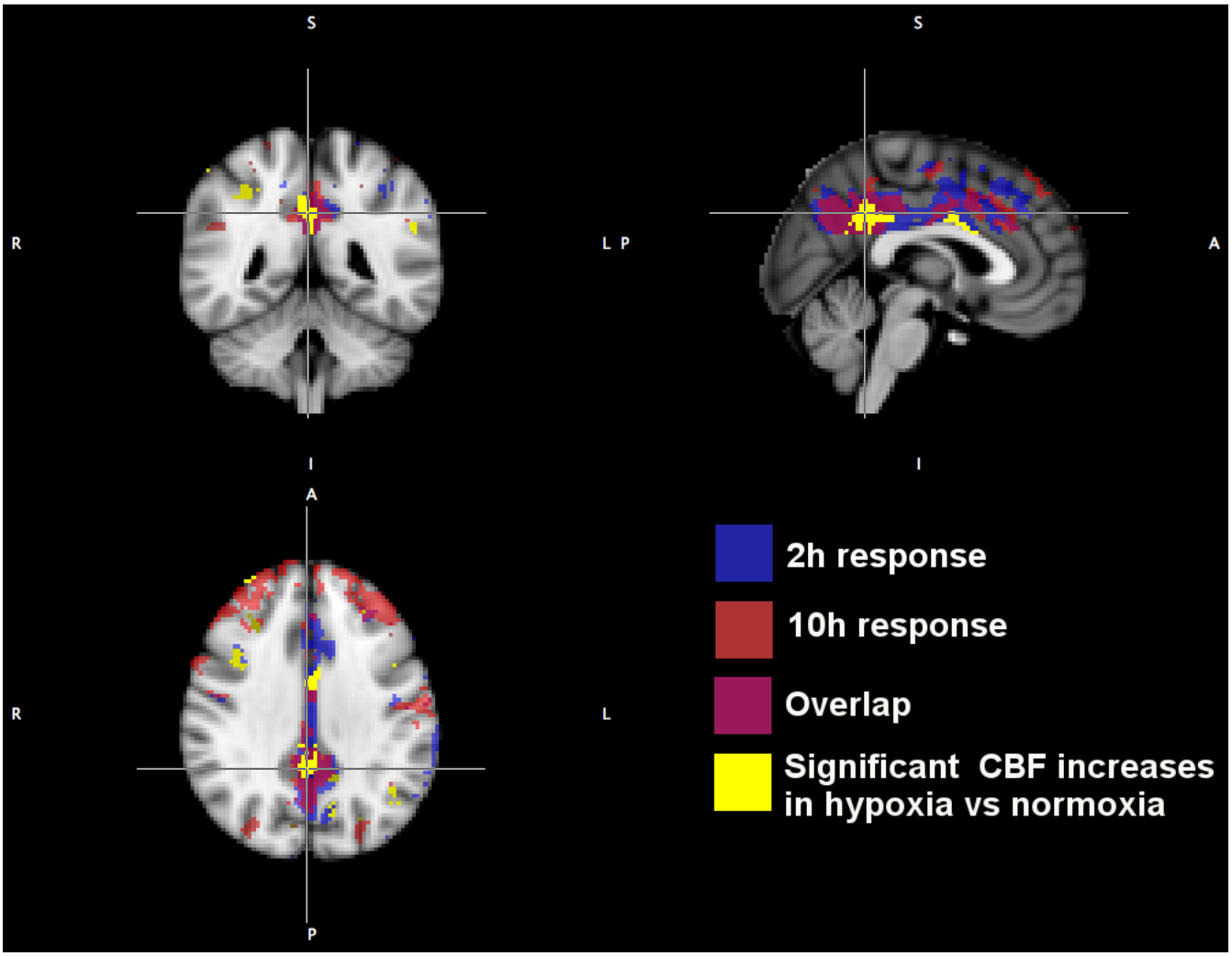

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