

# Isolating the independent effects of hypoxia and hyperventilation-induced hypocapnia on cerebral haemodynamics and cognitive function

Friend, Alexander; Balanos, George; Lucas, Sam

DOI:  
[10.1113/EP087602](https://doi.org/10.1113/EP087602)

License:  
Other (please specify with Rights Statement)

Document Version  
Peer reviewed version

Citation for published version (Harvard):  
Friend, A, Balanos, G & Lucas, S 2019, 'Isolating the independent effects of hypoxia and hyperventilation-induced hypocapnia on cerebral haemodynamics and cognitive function', *Experimental Physiology*, vol. 104, no. 10, pp. 1482-1493. <https://doi.org/10.1113/EP087602>

[Link to publication on Research at Birmingham portal](#)

**Publisher Rights Statement:**  
Checked for eligibility: 02/08/2019

This is the peer reviewed version of the following article: Friend, AT, Balanos, GM, Lucas, SJE. Isolating the independent effects of hypoxia and hyperventilation-induced hypocapnia on cerebral haemodynamics and cognitive function. *Experimental Physiology*. 2019, which has been published in final form at: <https://doi.org/10.1113/EP087602>. This article may be used for non-commercial purposes in accordance with Wiley Terms and Conditions for Use of Self-Archived Versions.

## General rights

Unless a licence is specified above, all rights (including copyright and moral rights) in this document are retained by the authors and/or the copyright holders. The express permission of the copyright holder must be obtained for any use of this material other than for purposes permitted by law.

- Users may freely distribute the URL that is used to identify this publication.
- Users may download and/or print one copy of the publication from the University of Birmingham research portal for the purpose of private study or non-commercial research.
- User may use extracts from the document in line with the concept of 'fair dealing' under the Copyright, Designs and Patents Act 1988 (?)
- Users may not further distribute the material nor use it for the purposes of commercial gain.

Where a licence is displayed above, please note the terms and conditions of the licence govern your use of this document.

When citing, please reference the published version.

## Take down policy

While the University of Birmingham exercises care and attention in making items available there are rare occasions when an item has been uploaded in error or has been deemed to be commercially or otherwise sensitive.

If you believe that this is the case for this document, please contact [UBIRA@lists.bham.ac.uk](mailto:UBIRA@lists.bham.ac.uk) providing details and we will remove access to the work immediately and investigate.

1 **Isolating the independent effects of hypoxia and hyperventilation-induced hypocapnia**  
2 **on cerebral haemodynamics and cognitive function.**

3 Alexander T. Friend<sup>1</sup>, George M. Balanos<sup>1</sup>, Samuel J.E. Lucas<sup>1,2</sup>

4 **Affiliations:**

5 *<sup>1</sup>School of Sport, Exercise and Rehabilitation Sciences, University of Birmingham,*  
6 *Edgbaston, UK*

7 *<sup>2</sup>Centre for Human Brain Health, University of Birmingham, Edgbaston, UK*

8

9 **Running title:** Cognitive and cerebrovascular responses to hypoxia and hypocapnia

10 **Keywords:** hypoxia, hypocapnia, cerebral blood flow, cognition

11 **Word count:** 6692 (Excluding references and figure legends)

12 **Reference Count:** 52

13 **Corresponding Author:**

14 Alexander Friend, Bangor University, George Building, Gwynedd, LL57 2PZ

15 alexander.friend@bangor.ac.uk

16 **Subject Area:** Environmental Physiology

17 **What is the central question of this study?**

18 To determine the independent effects of hypoxia and hypocapnia on cerebral haemodynamics  
19 and cognitive function.

20 **What is the main finding and its importance?**

21 Our data indicates that exposure to hyperventilation-induced hypocapnia causes cognitive  
22 impairment in both normoxia and hypoxia. In addition, supplementation of carbon dioxide  
23 during hypoxia alleviates the cognitive impairment and reverses hypocapnia-induced  
24 vasoconstriction of the cerebrovasculature. These data provide new evidence for the  
25 independent effect of hypocapnia on the cognitive impairment associated with hypoxia.

## Abstract

Hypoxia, which is accompanied by hypocapnia at altitude, is associated with cognitive impairment. This study examined the independent effects of hypoxia and hypocapnia on cognitive function and assessed how changes in cerebral haemodynamics may underpin cognitive performance outcomes. Single reaction time (SRT), five-choice reaction time (CRT) and spatial working memory (SWM) tasks were completed in 20 participants at rest and after one hour of isocapnic hypoxia (IH, end-tidal oxygen partial pressure ( $P_{ET}O_2$ ) = 45mmHg, end-tidal carbon dioxide partial pressure ( $P_{ET}CO_2$ ) clamped at normal), and poikilocapnic hypoxia (PH,  $P_{ET}O_2$  = 45mmHg,  $P_{ET}CO_2$  not clamped). A subgroup of 10 participants were also exposed to euoxic hypocapnia (EH,  $P_{ET}O_2$  = 100mmHg,  $P_{ET}CO_2$  clamped 8mmHg below normal). Middle cerebral artery velocity (MCAv) and prefrontal cerebral haemodynamics were measured with transcranial Doppler and near infrared spectroscopy, respectively. IH did not affect SRT and CRT performance from rest ( $566 \pm 50$ ms and  $594 \pm 70$ ms), whereas PH ( $721 \pm 51$ ms and  $765 \pm 48$ ms) and EH ( $718 \pm 55$ ms and  $755 \pm 34$ ms) slowed response times ( $p < 0.001$  vs IH). Performance on the SWM task was not altered by condition. MCAv increased during IH compared to PH ( $p < 0.05$ ), which was unchanged from rest. EH caused a significant fall in MCAv and prefrontal cerebral oxygenation ( $p < 0.05$  vs baseline). MCAv was moderately correlated to cognitive performance ( $R^2 = 0.266-0.289$ ), whereas prefrontal cerebral tissue perfusion and saturation were not ( $p > 0.05$ ). These findings reveal a role of hyperventilation-induced hypocapnia *per se* on the development of cognitive impairment during normoxic *and* hypoxic exposures.

27 Table of Abbreviations

28	CANTAB	Cambridge Neuropsychological Test Automated Battery
29	CaO <sub>2</sub>	arterial oxygen content
30	CBF	cerebral blood flow
31	CMRO <sub>2</sub>	cerebral metabolic rate of oxygen
32	CRT	five-choice reaction time task
33	EH	euoxic hypocapnia
34	HCO <sub>3</sub> <sup>-</sup>	bicarbonate ion
35	IE	isocapnic euoxic
36	IH	isocapnic hypoxia
37	MAP	mean arterial pressure
38	MCA <sub>v</sub>	middle cerebral artery velocity
39	NIRS	near infrared spectroscopy
40	nTHI	total haemoglobin normalised to the initial value
41	PaCO <sub>2</sub>	partial pressure of arterial carbon dioxide
42	PaO <sub>2</sub>	partial pressure of arterial oxygen
43	P <sub>ET</sub> CO <sub>2</sub>	end-tidal partial pressure of carbon dioxide
44	P <sub>ET</sub> O <sub>2</sub>	end-tidal partial pressure of oxygen
45	PH	poikilocapnic hypoxia
46	SRT	single reaction time task
47	SWM	spatial working memory
48	TCD	transcranial Doppler
49	TOI	total oxygenation index

50 Introduction

51 Exposure to high altitude can cause a number of hypoxia-induced physiological  
52 complications such as acute mountain sickness, pulmonary and/or cerebral oedema, and  
53 impairment of cognitive function (Hackett & Roach, 2001). Individuals become quickly  
54 aware of physical symptoms such as dizziness, headaches and nausea at altitude (Hackett &  
55 Roach, 2001), but they are less aware of the impairment to their cognitive function (Asmaro,  
56 Mayall, & Ferguson, 2013). The degree to which cognitive function is impaired is related to  
57 the severity of the hypoxic stimulus, particularly for tasks that require a higher order of  
58 cognitive ability (Petrassi, Hodkinson, Walters, & Gaydos, 2012; Yan, 2014). This higher  
59 order ability is essential for decision-making and attentional processes in individuals who  
60 venture to unfamiliar and dangerous environments, such as is typical of the high-altitude  
61 environment.

62 The brain relies on two variables to maintain sufficient oxygen supply and its functional  
63 capacity; namely, arterial oxygen content ( $CaO_2$ ) and cerebral blood flow (CBF). During  
64 exposure to hypoxia, partial pressure of arterial oxygen ( $PaO_2$ ) will fall (and related  $CaO_2$ )  
65 and subsequently the cerebrovasculature dilates in order to increase CBF to maintain global  
66 oxygen delivery to the brain (Kety & Schmidt, 1948; Willie, Tzeng, Fisher, & Ainslie, 2014).  
67 Simultaneously, the peripheral chemoreceptors activate the hypoxic ventilatory response to  
68 increase oxygen intake via the lungs. Consequently, this increased respiration gives rise to  
69 hypocapnia, a known vasoconstrictor of the cerebrovasculature (Kety & Schmidt, 1946).  
70 Therefore, the change in CBF is influenced by two conflicting stimuli, with the balance of  
71 these changes in oxygen and carbon dioxide tensions key factors in the overall change in  
72 CBF during exposure to hypoxia (Lucas et al., 2011; Bruce et al., 2016). Given this,  
73 hypocapnic-induced vasoconstriction could play a defining role in the cognitive impairment

74 experienced at altitude through compromising cerebral tissue perfusion via its effect on the  
75 capacity of the vasculature to dilate in response to hypoxaemia.

76 To investigate the physiological effects of hypocapnia participants are often instructed to  
77 voluntarily hyperventilate. Studies using this method have demonstrated that hypocapnia  
78 compromises brain function through its effect on the cerebrovasculature and produces similar  
79 impairment to that experienced at altitude, as evidenced by reports of light-headedness and  
80 dizziness (Bresseleers, Van Diest, De Peuter, Verhamme, & Van den Bergh, 2010), and  
81 impairment of complex cognitive tasks such as Stroop Test performance (Van Diest, Stegen,  
82 Van de Woestijne, Schippers, & Van den Bergh, 2000). The ambient gas compositions  
83 experienced at altitude are as consequence of a reduction in atmospheric pressure (hypobaric  
84 hypoxia), but can be mimicked in the laboratory setting through a reduction in partial  
85 pressure of oxygen (normobaric hypoxia). Despite some evidence suggesting different  
86 physiological responses between hypobaric hypoxia and normobaric hypoxia (Savoirey,  
87 Launay, Besnard, Guinet, & Travers, 2003), the ability to tightly control gas composition in  
88 the laboratory setting enables the comparison of poikilocapnic hypoxia (PH), as it occurs  
89 naturally from hypoxia-induced hyperventilation, to that of isocapnic hypoxia (IH), where the  
90 effects of hypoxia *per se* can be separated from hypocapnia by clamping partial pressure of  
91 arterial carbon dioxide ( $\text{PaCO}_2$ ) at its normal value. Using such an approach, Van Dorp et al.  
92 (2007) compared the effects of PH with that of IH on a combination of vigilance and multi-  
93 attribute cognitive tasks and found that carbon dioxide supplementation during hypoxia (IH)  
94 alleviated the impairment in cognitive function such that performance was similar to that  
95 under normoxic conditions. The authors concluded that the hypocapnic element of PH may  
96 be directly related to the compromised cognitive function.

97 However, the independent contribution of hypocapnia to cognitive function and its link to  
98 CBF during hypoxia remains unclear. To our knowledge, no study has attempted to separate

99 the roles of hypoxia *and* hypocapnia on cognitive function, as well as the associated changes  
100 in cerebral haemodynamics and task performance. Therefore, the present study was designed  
101 to examine the isolated effects of hypocapnia and hypoxia on simple and complex cognitive  
102 tasks, as well as to explore how changes in global and prefrontal cerebral haemodynamics  
103 might relate to changes in cognitive performance.

## 104 Methods

### 105 Ethical Approval

106 Ethical approval for this study was provided by the Safety and Ethics Subcommittee of the  
107 School of Sport, Exercise and Rehabilitation Sciences at the University of Birmingham  
108 (reference: MW 07/10/14) and was conducted in accordance with the standards of the  
109 *Declaration of Helsinki*, except for registration in a database, with written informed consent  
110 obtained from participants before they took part in the study.

### 111 Participants

112 Twenty young healthy males (aged  $22.4 \pm 6.3$  years) participated in this study. All  
113 participants completed a general health questionnaire and were invited to participate if they  
114 were healthy, non-smokers, and had no history of cardiorespiratory disease. Participants were  
115 asked to refrain from consuming alcohol and from undertaking strenuous exercise within 24  
116 hours of each experimental session. Participants were also asked not to consume caffeinated  
117 drinks within six hours, and food within two hours prior to reporting to the laboratory.

### 118 Study Design and Procedures

119 All participants visited the laboratory on three occasions, once for a familiarisation session  
120 and then for two experimental sessions performed in a random order and separated by at least  
121 48 hours. A subgroup of 10 participants completed a third experimental session. All



122 participants completed an IH session (end-tidal partial pressure of oxygen ( $P_{ET}O_2$ ) =  
123 45mmHg and end-tidal partial pressure of carbon dioxide ( $P_{ET}CO_2$ ) clamped at each  
124 participant's normal value) and a PH session ( $P_{ET}O_2$  = 45mmHg and  $P_{ET}CO_2$  not controlled),  
125 while the subgroup completed an additional euoxic hypocapnia (EH) session ( $P_{ET}O_2$  = 100  
126 mmHg and  $P_{ET}CO_2$  clamped at 8 mmHg below each participant's normal value) (see Figure  
127 2). Participants were blinded to IH and PH conditions only, as participants were coached to  
128 maintain a ventilation rate during EH.

### 129 *Familiarisation*

130 Participants visited the laboratory to familiarise themselves with the equipment and  
131 procedures that were used in the study. During this session, participants completed one repeat  
132 of the reaction time tasks and three repeats of the spatial working memory (SWM) task of the  
133 Cambridge Neuropsychological Test Automated Battery (CANTAB) programme to minimise  
134 any learning effect on performance outcomes during the experimental conditions.

### 135 *Isocapnic Hypoxia (IH)*

136 Participants were comfortably seated while being instrumented to measure cerebral  
137 haemodynamics, peripheral arterial oxygen saturation, mean arterial blood pressure and heart  
138 rate. The pulse oximeter probe and blood pressure finger cuff were attached to fingers on  
139 their non-dominant hand, allowing their dominant hand to be used for the cognitive function  
140 tests. Once instrumentation was complete and the signals were optimised, participants  
141 breathed through a mouthpiece whilst wearing a nose clip. Control of end-tidal gases was  
142 achieved by means of a dynamic end-tidal forcing system described in detail elsewhere  
143 (Robbins, Swanson, & Howson, 1982). Participants completed the first battery of cognitive  
144 function tests under isocapnic euoxic (IE) conditions ( $P_{ET}O_2$  = 100 mmHg and  $P_{ET}CO_2$   
145 clamped at participant's normal value). This was followed by a 60-minute intervention period

146 during which participants were exposed to IH, followed by a repeat of the cognitive function  
147 tests whilst remaining under IH conditions. Once the cognitive function tests were completed  
148 participants were returned to breathing room air and equipment was removed.

#### 149 *Poikilocapnic Hypoxia (PH)*

150 This protocol was identical to the one described for IH except that  $P_{ET}CO_2$  was not controlled  
151 during the 60-minute intervention and during the repeat of the cognitive function tests.

#### 152 *Euoxic Hypocapnia (EH, n=10)*

153 This protocol was identical to the one described for IH except that participants were exposed  
154 to EH during the 60-minute intervention and during the repeat of the cognitive function tests.  
155 Hypocapnia was achieved through voluntary hyperventilation. For this, participants were  
156 coached to hyperventilate enough to reduce their  $P_{ET}CO_2$  to approximately 10 mmHg below  
157 their normal value, allowing the dynamic end-tidal forcing system to then adjust  $P_{ET}CO_2$  to 8  
158 mmHg below accurately. Figure 1 shows a schematic of the protocol during each  
159 experimental visit, as well as examples of each of the CANTAB tests completed under each  
160 condition.

#### 161 Equipment and Measures

#### 162 Cognitive Function Assessment

163 Cognitive function was measured via a touch screen CANTAB cognition computer test  
164 (Cambridge Cognition Ltd., United Kingdom). The CANTAB is a valid neuropsychological  
165 testing instrument of cognitive function (Smith, Need, Cirulli, Chiba-Falek, & Attix, 2013),  
166 and is regularly used to assess cognitive function in both healthy and neurodegenerative  
167 cohorts (e.g. mild cognitive impairment (Saunders & Summers, 2010) and Alzheimer's

168 disease (Matos Goncalves, Pinho, & Simoes, 2018)). Reaction time tasks and the SWM tests  
169 were performed representing simple and complex cognitive tasks respectively.

### 170 *Reaction Time Tasks*

171 Reaction time was measured through two tasks; single reaction time task (SRT) and five-  
172 choice reaction time task (CRT). Both tasks required participants to hold down a pressure pad  
173 placed in front of the computer and to tap a circle on the monitor as quickly as possible after  
174 a yellow spot was displayed within it. The time taken for the yellow spot to appear was  
175 randomised between trials. This task was completed with a single response circle for the  
176 SRT, whilst the spot had the option to flash in any one of five response circles in the CRT  
177 (see Figure 1a). Participants were given practice attempts of both tasks prior to the test period  
178 in which their performance was recorded. Performance time was recorded as the sum of  
179 reaction time (time taken between the yellow spot appearing and releasing the pad) and  
180 movement time (time taken between releasing the pad and tapping the circle). Additionally,  
181 error count (releasing the pad too early or missing the correct circle) was measured for both  
182 reaction time tasks.

### 183 *Spatial Working Memory Task*

184 SWM was measured through a visuospatial task. The participant was presented with a  
185 selection of coloured boxes on the screen and the aim was to find all of the tokens hidden  
186 inside these boxes. Participants were required to use working memory and a process of  
187 elimination to find all of the tokens as only one token was hidden at a time and would never  
188 be found in the same box again. Three sets of practice trials (three boxes within each set)  
189 were completed before performance was recorded across three stages of increasing difficulty,  
190 with each stage consisting of four sets of trials with four, six and eight boxes, respectively,  
191 for each level of increasing difficulty. The total number of errors were recorded as the

192 measure of performance. Errors were recorded when participants returned to a box where a  
193 token had already been found, or when a box that had been previously selected was selected  
194 again in a subsequent search.

#### 195 Cerebral blood flow velocity and prefrontal cerebral haemodynamics

196 Bilateral measures of blood flow velocity from the left and right middle cerebral artery  
197 (MCAv) were measured using a 2 MHz pulsed Transcranial Doppler (TCD) ultrasound  
198 system (Doppler Box, DWL, Compumedics Ltd, Germany) using standardised procedures  
199 (Willie et al., 2011). Probes were placed over the left and right temporal windows and  
200 secured in place via an adjustable head piece. Photographs of the probe position and angle  
201 were used to replicate the placement between sessions, and signal depth and gain settings  
202 were also replicated. Left and right side MCAv measures were averaged, reported as a pooled  
203 mean, and expressed as a change from resting baseline.

204 In the subgroup of 10 participants that completed all three protocols prefrontal cerebral  
205 haemodynamics was also monitored non-invasively on the left and right side of the forehead  
206 using near infrared spectroscopy (NIRS; NIRO-200NX, Hamamatsu Photonics KK;  
207 Hamamatsu, Japan). The NIRS probes were housed in light-shielding cases and attached to  
208 the forehead skin with tape in the same position for each session. Probes were placed as  
209 lateral and superior as possible to avoid the frontal sinus and to allow the TCD head piece to  
210 fit between the probes and the superior orbital ridge (i.e. probe centre points were located  
211 approximately 4 cm from the midline and approximately 3 cm above the orbital ridge). The  
212 NIRO-200NX device measures changes in chromophore concentrations of oxyhaemoglobin  
213 and deoxyhaemoglobin via the modified Beer-Lambert law and provides depth-resolved  
214 measures of tissue oxygen saturation [total oxygenation index (TOI)] and tissue haemoglobin  
215 content (i.e., relative value of the total haemoglobin normalised to the initial value, nTHI)

216 using the spatially resolved spectroscopy (SRS) method. The SRS-derived NIRS parameters  
217 limit contamination from superficial tissue via depth-resolved algorithmic methods, providing  
218 an index of targeted local tissue saturation (TOI) and perfusion (nTHI) (Davies et al., 2015).  
219 Given the inter-individual variability of baseline measures using this imaging technology  
220 (Davies et al., 2017) and in accordance with recommendations of others (Subudhi, Miramon,  
221 Granger, & Roach, 2009), these NIRS data are expressed as the magnitude of the change  
222 from the resting baseline value.

223 Cerebrovascular haemodynamics, cardiovascular and respiratory variables were all acquired  
224 continuously at 200 Hz using an analogue-to-digital converter (Powerlab/16SP ML795;  
225 ADInstruments, New Zealand) interfaced and displayed in real time using LabChart software  
226 (Chart v7.5, ADInstruments) on a computer.

#### 227 Data Analysis

228 Mean SRT and CRT performance time and error count, and SWM task mean error count  
229 were collected from each CANTAB trial. A 60 s mean for MCA<sub>v</sub>, TOI and nTHI data were  
230 collected from the two baseline measures that preceded each CANTAB battery under IE or  
231 experimental conditions. During CANTAB battery periods, MCA<sub>v</sub>, TOI and nTHI data were  
232 averaged from the final 20 s of each reaction time task (SRT and CRT) and the final 30 s of  
233 the SWM task. One participant's TOI data was lost due to corruption of the containing file.

234 A repeated-measures analysis of variance (IBM SPSS Statistics v23) was used to assess the  
235 relations between condition (IH, PH, EH), time (IE, Experimental) and task phase (Baseline,  
236 SRT, CRT, SWM) for each physiological variable. A repeated measures analysis of variance  
237 was also used to assess the relations between condition (IH, PH, EH) and time (IE,  
238 Experimental) for each CANTAB performance variable. Pairwise comparisons (Bonferroni  
239 adjusted) were applied to evaluate main effects and interactions. The relationship between

240 changes in selected physiological variables (MCA<sub>v</sub>, TOI, nTHI) and change in reaction time  
241 task performance (SRT and CRT) were determined using Pearson's correlations. Data are  
242 presented as mean ± SD and statistical significance was accepted at  $p < 0.05$ .

## 243 Results

244 There were marked differences between IE baseline and experimental measures of P<sub>ET</sub>O<sub>2</sub>,  
245 P<sub>ET</sub>CO<sub>2</sub>, MCA<sub>v</sub>, TOI and nTHI (see Table 1). This general pattern was consistent during  
246 cognitive testing (see Figures 2 and 3), with no significant differences between the measured  
247 time points within each condition (all  $p > 0.05$ ). Nevertheless, we have presented the  
248 haemodynamics for each specific time point in Figure 3, but for brevity we have summarised  
249 our findings using pooled data across the cognitive tasks and report differences between  
250 condition (IH, PH, EH) and time (IE, Experimental) for each dependent variable.

## 251 End Tidal Gas Control

252 Baseline and experimental end-tidal values are shown in Table 1, and a representative  
253 example of the differences shown in Figure 2. By design, end-tidal gases were similar during  
254 IE conditions, and were successfully manipulated and held consistent during cognitive testing  
255 under experimental conditions. Specifically, P<sub>ET</sub>CO<sub>2</sub> remained clamped at IE values during  
256 IH ( $41.1 \pm 2.0$  mmHg), whereas P<sub>ET</sub>CO<sub>2</sub> declined during the PH ( $37.4 \pm 2.7$  mmHg;  $p <$   
257  $0.001$  vs IE and  $p < 0.001$  vs IH). For the subgroup completing the EH condition, P<sub>ET</sub>CO<sub>2</sub>  
258 was lowered to  $32.6 \pm 2.3$  mmHg ( $p < 0.001$  vs IE), significantly lower than IH ( $40.9 \pm 1.8$   
259 mmHg;  $p < 0.001$ ) and PH ( $36.6 \pm 3.0$  mmHg;  $p < 0.01$ ). The reductions in P<sub>ET</sub>O<sub>2</sub> during IH  
260 ( $44.2 \pm 1.7$  mmHg) and PH ( $43.2 \pm 2.4$  mmHg) interventions were similar (both  $p < 0.05$  vs  
261 IE). For the subgroup, P<sub>ET</sub>O<sub>2</sub> during the EH condition remained clamped at IE levels ( $98.6 \pm$   
262  $6.0$  mmHg), which was significantly greater than IH ( $44.2 \pm 2.2$  mmHg;  $p < 0.001$ ) and PH  
263 ( $43.5 \pm 3.2$  mmHg;  $p < 0.001$ ).

## 264 Haemodynamic Measurements (Isocapnic Euoxic vs Experimental conditions)

265 Baseline absolute measures of heart rate, mean arterial pressure (MAP), MCAv, nTHI and  
266 TOI in IE conditions were consistent between all sessions and are shown in Table 1. There  
267 was no difference in heart rate between IE and experimental conditions, whereas there was a  
268 main effect of time for MAP ( $p < 0.05$ ) representing elevated values during the experimental  
269 conditions compared to IE baseline. Compared to IE, MCAv increased during IH (up  $6.7 \pm$   
270  $7.2 \text{ cm}\cdot\text{s}^{-1}$ ;  $p < 0.001$  vs IE) whereas it remained similar during PH ( $p = 0.63$  vs IE) and thus  
271 lower than IH ( $p < 0.001$ ). In the subgroup, similar results for IH (up  $6.6 \pm 8.5 \text{ cm}\cdot\text{s}^{-1}$ ;  $p <$   
272  $0.05$  vs IE) and PH ( $p = 0.16$  vs IE, and  $p < 0.05$  vs IH) conditions were seen, while MCAv  
273 decreased by  $9.2 \pm 6.4 \text{ cm}\cdot\text{s}^{-1}$  from IE ( $p < 0.001$ ) during the EH condition ( $p < 0.01$  vs IH,  
274 and  $p = 0.18$  vs PH). Measures of prefrontal cerebral haemodynamics collected via NIRS in  
275 the subgroup completing all three conditions demonstrated that prefrontal perfusion (as  
276 indexed by nTHI) increased from IE for IH (up  $0.05 \pm 0.05$  au;  $p < 0.05$  vs IE) and PH (up  
277  $0.05 \pm 0.08$  au;  $p = 0.071$  vs IE), while nTHI decreased in EH (down  $0.05 \pm 0.04$  au;  $p < 0.05$   
278 vs IE, and  $p < 0.05$  IH vs PH). All conditions recorded a significant decline in prefrontal  
279 tissue saturation (indexed by TOI) compared to IE ( $p < 0.001$ ), with a greater decrease  
280 recorded in IH (down  $8.8 \pm 3.2\%$ ) and PH (down  $9.4 \pm 3.3\%$ ) conditions relative to EH  
281 (down  $4.2 \pm 2.0\%$ ;  $p < 0.05$  vs IH and PH). Figure 3 shows these cerebral haemodynamic  
282 changes for each experimental condition relative to the preceding IE baseline.

## 283 Cognitive Task Performance

284 *Simple and Complex Reaction Time:* Performance scores for both reaction time tasks are  
285 shown in Table 2. Baseline IE measures were consistent between all conditions ( $p > 0.05$ ).  
286 During IH, performance times for SRT ( $566 \pm 50$  ms) and CRT ( $594 \pm 70$  ms) tasks were  
287 unaffected with respect to IE ( $p > 0.05$ ), whereas PH caused a significant slowing of both

288 SRT (by  $149 \pm 81$  ms;  $p < 0.001$  vs IH) and CRT (by  $152 \pm 82$  ms;  $p < 0.001$  vs IH)  
289 performance. For the subgroup, EH produced similar performance decrements as was  
290 observed during PH ( $p > 0.05$ ) for both SRT (slower by  $174 \pm 42$  ms;  $p < 0.001$  vs IH) and  
291 CRT (slower by  $167 \pm 70$  ms;  $p < 0.001$  vs IH) performance. There was no effect of condition  
292 on SRT and CRT error count.

293 *Spatial Working Memory Task:* Error count for the SWM task is shown in Table 2. There was  
294 no significant change in error count during the experimental conditions compared to IE  
295 conditions for any protocols.

#### 296 Relation between cerebral haemodynamics and cognitive task performance

297 Finally, as shown in figure 4A and B, changes in MCAv were moderately correlated ( $R^2 =$   
298  $\sim 0.28$ ) with both SRT and CRT, such that increases in MCAv were associated with  
299 maintained reaction time task performance. These correlations were not apparent in the  
300 NIRS-derived prefrontal cortex measures of tissue saturation and perfusion (as indexed by  
301 TOI and nTHI, respectively), with no significant correlations observed (all  $p > 0.05$ , see  
302 Figures 4C-F).

#### 303 Discussion

304 The present study was designed to investigate the independent roles of hypoxia and  
305 hypocapnia on simple and complex cognitive ability, and how changes in global and  
306 prefrontal cerebral haemodynamics were associated with altered cognitive performance. We  
307 found that acute exposure to PH impaired both SRT and CRT performance, but it had no  
308 apparent effect on SWM task performance. Hypocapnia alone (i.e. EH) produced similar  
309 decrements to those seen during PH, whilst the supplementation of carbon dioxide to  
310 maintain  $P_{ET}CO_2$  relieved the hypoxia-induced cognitive impairment. The associated changes  
311 in cerebral haemodynamics indicate that differences in CBF between the experimental  
312 conditions may mediate this effect, with the changes in global flow (as indexed by MCAv)



313 moderately correlated to cognitive task performance. Interestingly, despite differences in  
314 global flow and the associated link to performance, prefrontal cerebral tissue perfusion and  
315 saturation were not different between hypoxic trials and not linked to cognitive performance.  
316 Overall, these findings reveal a significant role of hypocapnia *per se* on the development of  
317 cognitive impairment during normoxic *and* hypoxic exposures.

### 318 Cognitive Function during Hypoxia and Hypocapnia

319 The observed detriment to cognitive function during PH reported in the current study is  
320 consistent with previous work showing impairment in CRT during exposure to high altitude  
321 (Dykiert et al., 2010). Further, our findings of the recovered cognitive performance during  
322 carbon dioxide supplementation in hypoxia has also been previously demonstrated (Van Dorp  
323 et al., 2007). However, to our knowledge no such cognitive impairment has been found when  
324 tasks are completed under hypocapnia when controlling for hypoxia, nor demonstrated how  
325 cerebral haemodynamic changes may mediate this effect (discussed below). Interestingly,  
326 Bloch-Salisbury and colleagues reported significant changes to electroencephalographic  
327 signals under hypocapnia during a series of rapid-response cognitive tasks (Bloch-Salisbury,  
328 Lansing, & Shea, 2000); however, these changes did not reflect impairment to response time  
329 or error scores despite a similar hypocapnic stimulus ( $P_{ET}CO_2$  of  $\sim 30$ mmHg) to that induced  
330 in the current study. The present data exhibited a speed-accuracy trade-off for SRT and CRT  
331 performance during PH and EH conditions, with significantly slower performance times  
332 recorded with no change to error count. An unexpected finding of the present study was that  
333 the performance of the SWM task was unaffected by all conditions. It is widely accepted that  
334 as altitude increases, complex cognitive abilities, such as working memory become  
335 progressively impaired (reviewed in Yan, 2014). Studies using test batteries to examine  
336 executive function performance during hypoxia have found impairments in the Paced  
337 Auditory Serial Addition Test (PASAT) (Malle et al., 2013) and Stroop Test (Turner, Barker-

338 Collo, Connell, & Gant, 2015) task performance. Despite differences in mean average error  
339 count, it is likely that we did not have the power (effect size = 0.279, observed power =  
340 0.498) to detect any significant differences in SWM task performance as a consequence a  
341 lack of sensitivity of the SWM CANTAB task. Further, Lowe and Rabbitt (1998) described  
342 that for executive function to be measured effectively tasks must remain novel to the  
343 participant due to the rapid improvements in performance once an optimal strategy is  
344 discovered. Specifically, the familiarisation session conducted to minimise the learning  
345 effects may have provided a ceiling effect for SWM task performance. The CANTAB SWM  
346 task used here is designed to test memory retention, strategy, and visuospatial abilities as a  
347 representation of executive function. The version of the SWM task used in this present study  
348 produces 15 identical arrangements of coloured boxes for each repeat, which may diminish  
349 its ability to reliably measure executive function. Patients with mild cognitive impairment  
350 and Alzheimer's disease completing the CANTAB SWM task in a 6 month test-retest  
351 assessment are shown to exhibit a practice effect by optimising their strategy search patterns,  
352 which was maintained at the 12-month re-test assessment (Cacciamani et al., 2018).  
353 Subsequently in the present study, the acute test-retest period that was used (within ~1 hr)  
354 would likely have been compromised by this learning effect. In addition, the measurements  
355 of error collected by the SWM task may not provide adequate information to determine  
356 whether there is impairment to performance. Based on our reaction time task performance  
357 decrements, it was the speed of the response that was impaired as opposed to the accuracy.  
358 As such, including a time pressure during a cognitive task may be a more effective way to  
359 demonstrate the hypoxic impairment effect given its effect on a recall task (Earles, Kersten,  
360 Berlin Mas, & Miccio, 2004). Indeed, this is consistent with observations of hypoxia-related  
361 impairment of PASAT test performance (Malle et al., 2013), a task which includes a time  
362 pressure.

363 Cerebral Haemodynamics and Cognitive Function

364 Exposure to hypoxia is well known to cause a cerebral vasodilatory response but is  
365 compromised by the reflex hypoxia-induced hyperventilation response lowering PaCO<sub>2</sub> and  
366 causing cerebral vasoconstriction (Ainslie & Ogoh, 2010). In the present study, there was no  
367 change in MCAv observed during PH, reflecting the contrasting cerebrovascular activity that  
368 hypoxia and hypocapnia stimulate (Mardimae et al., 2012). Consistent with previous  
369 observations, the supplementation of carbon dioxide to maintain P<sub>ET</sub>CO<sub>2</sub> constant during the  
370 hypoxic exposure (i.e. IH) allowed the cerebrovasculature to dilate and thus to increase  
371 oxygen delivery to the brain via elevated flow (Van Dorp et al., 2007). Indeed, higher blood  
372 flow velocity was associated with maintained reaction time task performance (Fig 4A and  
373 4B). Interestingly, while increases in global cerebral haemodynamics were observed during  
374 IH compared to PH (and EH), the NIRS-based measures of regional tissue perfusion as  
375 indexed by haemoglobin content (i.e. nTHI) at the prefrontal cortex was not different  
376 between the hypoxia conditions, which increased similarly in both hypoxic conditions. A  
377 potential explanation is that this may reflect a global increase in CBF during IH, compared to  
378 a regional shift of blood towards active areas of the brain during PH, particularly at the  
379 prefrontal cortex. Binks and colleagues reported a global increase in CBF to all areas of the  
380 brain during IH, but not necessarily each to the same magnitude (Binks, Cunningham,  
381 Adams, & Banzett, 2008). Additionally, Lawley et al. reported an active heterogeneous CBF  
382 response following two hours of PH, with increased perfusion observed in the anterior  
383 portions of the brain and reductions to the posterior regions (Lawley, Macdonald, Oliver, &  
384 Mullins, 2017). It is known that different portions of the brain are activated depending on the  
385 task completed, with working memory processes stimulating the prefrontal cortex (van  
386 Asselen et al., 2006), whereas reaction time tasks activate both the premotor and primary  
387 sensorimotor areas (Kwon, Kwon, & Park, 2013). This regional activation may explain why

388 no significant haemodynamic differences were seen between the impaired reaction time tasks  
389 and the unimpaired SWM task as only prefrontal cortex measurements were recorded.  
390 Further investigation using whole-head functional imaging would enable a clearer  
391 understanding of the regional differences in CBF during cognitive tasks under hypoxia and  
392 hypocapnia.

393 Despite possible differences in the maintenance of local blood flow, there was an equivalent  
394 fall in cerebral oxygenation (TOI) observed in both IH and PH, indicating that insufficient  
395 delivery of oxygen to the tissue is not the defining factor behind the cognitive function  
396 difference. This is demonstrated with no meaningful correlations found between TOI and  
397 reaction time task performance (Fig 4C-D). Hypocapnia causes haemoglobin to have an  
398 increased affinity for oxygen and reduce oxygen unloading at the tissues (Collins, Rudenski,  
399 Gibson, Howard, & O'Driscoll, 2015). This may be a defining factor between the two  
400 hypoxic conditions in the development of cognitive impairment, with the supplementation of  
401 carbon dioxide reversing the leftward shift of the oxygen-haemoglobin dissociation curve,  
402 allowing adequate offloading of oxygen into the tissue. This is highlighted during EH given  
403 that there was less of a fall in TOI but still a cognitive impairment. With hypoxia-induced  
404 hypocapnia comes respiratory alkalosis and acid-base adjustment via renal compensation  
405 through excretion of bicarbonate ion ( $\text{HCO}_3^-$ ), although this is typically reported with longer  
406 exposures than the 60 minutes we used here. Further, it remains undefined whether  $\text{PaCO}_2$  or  
407 pH acts as the primary stimulant responsible for cerebral vasoconstriction (Willie, Tzeng,  
408 Fisher, & Ainslie, 2014). Nonetheless, hypocapnia-induced vasoconstriction has been shown  
409 to impact the neurovascular coupling response, such that it overwhelms the neuronal  
410 activated vasodilation response to visual stimulation, and compromises oxygen supply to the  
411 brain (Szabo et al., 2011). The combination of a compromised oxygen supply and reduced  
412 oxygen unloading causes hypocapnia-induced brain ischaemia (Laffey & Kavanagh, 2002)

413 and could well be an underlying factor in the development of the cognitive impairment during  
414 hypoxic exposure. In addition to altering the neurovascular coupling response, the cerebral  
415 metabolic rate of oxygen (CMRO<sub>2</sub>) does not change during isocapnic hypoxia (Ainslie et al.,  
416 2014), with MRI-based evidence indicating that increased neural excitability (and subsequent  
417 CMRO<sub>2</sub>) during hypoxia are as a consequence of hypoxic ventilatory response-induced  
418 hypocapnia (Smith et al., 2012; Vestergaard et al., 2015). This increase in CMRO<sub>2</sub> has been  
419 shown to be mitigated during hypoxia with the administration of acetazolamide (Wang,  
420 Smith, Buxton, Swenson, & Dubowitz, 2015), which indicates an important role of  
421 hypocapnia and alkalosis in cerebral metabolism during acute hypoxia.

422 In the present study, the use of an acute exposure to normobaric hypoxia enables the  
423 controlled manipulation of oxygen and carbon dioxide to investigate their impact on  
424 cognitive function. During extended or chronic exposures to hypobaric hypoxia (i.e. the  
425 natural high-altitude environment), a complex integrative response to hypoxia will also  
426 include haematological and extended nephrological compensation in addition to regulation by  
427 arterial blood gases. Consequently, the effect of respiratory alkalosis on CBF, metabolism  
428 and cognitive function is likely to be influenced by the degree of hypoxic ventilatory  
429 response and renal compensation during acclimatization. Similarly, haemoglobin increases  
430 occur within weeks of high altitude exposure and improve CaO<sub>2</sub> and global oxygen delivery  
431 (Subudhi et al., 2014). Therefore, cognitive impairment to tasks involving sustained attention  
432 (i.e. tasks involving reaction time) often occur during the initial exposure to high altitude  
433 (4,350m and 5,050m), but are reversed within the days following acclimatization (Davranche  
434 et al., 2016; Pun et al., 2018).

#### 435 Methodological Considerations

436 An important consideration to acknowledge is that during EH  $P_{ET}CO_2$  was not matched to the  
437 changes in  $P_{ET}CO_2$  induced during PH (i.e.  $P_{ET}CO_2$  significantly different between PH and  
438 EH conditions). Our aim was to elicit a hypocapnic state that resembled the one that results  
439 from the natural hyperventilation caused by hypoxia, but in reality we overestimated this  
440 response when selecting the target  $P_{ET}CO_2$  in EH. This could have been avoided if all  
441 participants had undertaken the EH condition after the PH condition, but of course this would  
442 then introduce a problematic order effect. Nevertheless, studies report that there is a linear  
443 graded response of cerebral saturation with carbon dioxide tensions (Mutch et al., 2013), and  
444 so mechanisms by which hypocapnia induces cognitive impairment may also work in a  
445 graded fashion.

446 Active hyperventilation is attention consuming when compared to passive hyperventilation  
447 (Gallego, Perruchet, & Camus, 1991), and subsequently may confound any interpretation of  
448 hypocapnia on cognitive functioning. To overcome this, previous studies have assessed  
449 cognitive function during the minutes of recovery from hyperventilation-induced hypocapnia  
450 (Van Diest et al., 2000). However, our battery of cognitive tasks took approximately 15  
451 minutes to complete, which was too long to use such an approach. Indeed, Malatino and  
452 colleagues demonstrated that  $MCA_v$  returns to near baseline values within five minutes  
453 following hyperventilation-induced hypocapnia, and this was from a greater level of  
454 hypocapnia ( $P_{ET}CO_2=20$  mm Hg) than induced in the current study (Malatino et al., 1992).  
455 Nonetheless, completing a normocapnic normoxic hyperventilation trial would determine the  
456 effect of active hyperventilation on the cognitive function. Transcranial Doppler measures  
457 blood flow velocity as an index of vessel blood flow based on the assumption that the  
458 diameter of the MCA remains constant. This assumption has recently been questioned  
459 (Ainslie & Hoiland, 2014) and evidence for altered MCA diameter in conditions where blood  
460 gas content is affected has been demonstrated (Coverdale, Gati, Opalevych, Perrotta, &

461 Shoemaker, 2014; Verbree et al., 2014; Wilson et al., 2011). Nevertheless, if the diameter of  
462 the MCA was increased (in IH) or decreased (EH) as a consequence of the manipulated blood  
463 partial pressures, our TCD-based findings would only underestimate the true effect observed  
464 here.

465 As mentioned above, we only measured prefrontal cerebral haemodynamic changes with our  
466 NIRS and so the regional perfusion shifts proposed would need to be confirmed via whole-  
467 head NIRS imaging (or with functional magnetic resonance imaging). Further, NIRS is  
468 limited to the cortex surface and currently available technology and analysis approaches does  
469 not differentiate between skin and skull blood flow, and cerebrospinal fluid. However,  
470 despite its spatial limitations NIRS is clearly able to measure changes in haemodynamic  
471 responses, and which are more likely to result from neural activation than haemoglobin  
472 content shift within the blood vessels of the skin under this experimental paradigm (Davies et  
473 al., 2016; Davies et al., 2017). Finally, these apparatuses only reflect global CBF and regional  
474 haemoglobin content, representing vascular flow and oxygenation changes to our measured  
475 areas of interest. Neither of these imaging devices provided any measure of cerebral  
476 metabolic rate of oxygen, which may better reflect the mechanisms of cognitive (dys)function  
477 during hypoxia and hypocapnia exposure and warrants future study.

## 478 Conclusion

479 Hyperventilation-induced hypocapnia impairs performance of simple and five-choice reaction  
480 time tasks during normoxia and hypoxia, but not working memory cognitive performance.  
481 Furthermore, supplementation of carbon dioxide during hypoxia preserved cognitive function  
482 and facilitates an appropriate vascular response. The associated changes in global cerebral  
483 haemodynamics between the experimental conditions may mediate this effect, with the  
484 changes in MCAv moderately correlated to cognitive task performance. Taken together, these

485 findings reveal the significant role of hypocapnia *per se* on the development of cognitive  
486 impairment during normoxic *and* hypoxic exposures.



487 **Author Contributions:** GB and SL conceived and designed the study. All authors  
488 contributed to the acquisition, analysis, or interpretation of data for the work. AF and SL  
489 drafted the manuscript, with GB reviewing and providing critical feedback important for  
490 intellectual content. All authors have approved the final version of the paper and agree to be  
491 accountable for all aspects of the work in ensuring that questions related to the accuracy or  
492 integrity of any part of the work are appropriately investigated and resolved. All persons  
493 designated as authors qualify for authorship, and all those who qualify for authorship are  
494 listed.

545 **Acknowledgements:** We would like to thank the participants for their time and effort in this  
546 study. We also thank Alex Peart, Shaun Webster, Matthew Holloway, Jamie Phillips,  
547 Timothy Riviere, Sebastian Cox, Ciaran O’Connell, Daniel Slater, Josh Kelly and Patrick  
548 Gravett-Curl for their contribution to data collection.

549 **Funding:** No funding received.

550 **Conflict of interest:** There authors declare that they have no conflicts of interest.

551 References

- 552 Ainslie, P. N., & Hoiland, R. L. (2014). Transcranial Doppler ultrasound: Valid, invalid, or  
553 both? *Journal of Applied Physiology*, *117*(10), 1081–1083.  
554 <https://doi.org/10.1152/jappphysiol.00854.2014>
- 555 Ainslie, P. N., & Ogoh, S. (2010). Regulation of cerebral blood flow in mammals during  
556 chronic hypoxia: a matter of balance. *Exp Physiol*, *95*(2), 251–262.  
557 <https://doi.org/10.1113/expphysiol.2008.045575>
- 558 Ainslie, P. N., Shaw, A. D., Smith, K. J., Willie, C. K., Ikeda, K., Graham, J., & Macleod, D.  
559 B. (2014). Stability of cerebral metabolism and substrate availability in humans during  
560 hypoxia and hyperoxia. *Clinical Science*, *126*(9), 661 LP – 670.  
561 <https://doi.org/10.1042/CS20130343>
- 562 Asmaro, D., Mayall, J., & Ferguson, S. (2013). Cognition at altitude: impairment in executive  
563 and memory processes under hypoxic conditions. *Aviat Space Environ Med*, *84*(11),  
564 1159. <https://doi.org/10.3357/ASEM.3661.2013>
- 565 Binks, A. P., Cunningham, V. J., Adams, L., & Banzett, R. B. (2008). Gray matter blood flow  
566 change is unevenly distributed during moderate isocapnic hypoxia in humans. *J Appl*  
567 *Physiol* (1985), *104*(1), 212–217. <https://doi.org/10.1152/jappphysiol.00069.2007>
- 568 Bloch-Salisbury, E., Lansing, R., & Shea, S. A. (2000). Acute changes in carbon dioxide  
569 levels alter the electroencephalogram without affecting cognitive function.  
570 *Psychophysiology*, *37*(4), 418–426.
- 571 Bresseleers, J., Van Diest, I., De Peuter, S., Verhamme, P., & Van den Bergh, O. (2010).  
572 Feeling lightheaded: the role of cerebral blood flow. *Psychosom Med*, *72*(7), 672–680.  
573 <https://doi.org/10.1097/PSY.0b013e3181e68e94>
- 574 Bruce, C. D., Steinback, C. D., Chauhan, U. V., Pfoh, J. R., Abrosimova, M., Vanden Berg, E.  
575 R., ... Day, T. A. (2016). Quantifying cerebrovascular reactivity in anterior and  
576 posterior cerebral circulations during voluntary breath holding. *Experimental*  
577 *Physiology*, *101*(12), 1517–1527. <https://doi.org/10.1113/EP085764>
- 578 Cacciamani, F., Salvadori, N., Eusebi, P., Lisetti, V., Luchetti, E., Calabresi, P., & Parnetti,  
579 L. (2018). Evidence of practice effect in CANTAB spatial working memory test in a  
580 cohort of patients with mild cognitive impairment. *Applied Neuropsychology. Adult*,  
581 *25*(3), 237–248. <https://doi.org/10.1080/23279095.2017.1286346>
- 582 Collins, J. A., Rudenski, A., Gibson, J., Howard, L., & O’Driscoll, R. (2015). Relating  
583 oxygen partial pressure, saturation and content: the haemoglobin-oxygen dissociation  
584 curve. *Breathe (Sheff)*, *11*(3), 194–201. <https://doi.org/10.1183/20734735.001415>
- 585 Coverdale, N. S., Gati, J. S., Opalevych, O., Perrotta, A., & Shoemaker, J. K. (2014).  
586 Cerebral blood flow velocity underestimates cerebral blood flow during modest  
587 hypercapnia and hypocapnia. *Journal of Applied Physiology*, *117*(10), 1090–1096.  
588 <https://doi.org/10.1152/jappphysiol.00285.2014>
- 589 Davies, D., Evans, S., Clancy, M., Su, Z., Hansen, P., Dehghani, H., ... Lucas, S. (2016).  
590 Comparison of near infrared spectroscopy with functional MRI for detection of  
591 physiological changes in the brain independent of superficial tissue. *The Lancet*, *387*,

- 592 S34. [https://doi.org/10.1016/S0140-6736\(16\)00421-9](https://doi.org/10.1016/S0140-6736(16)00421-9)
- 593 Davies, D. J., Clancy, M., Lighter, D., Balanos, G. M., Lucas, S. J. E., Dehghani, H., ... Belli,  
594 A. (2017). Frequency-domain vs continuous-wave near-infrared spectroscopy devices: a  
595 comparison of clinically viable monitors in controlled hypoxia. *J Clin Monit Comput*,  
596 *31*(5), 967–974. <https://doi.org/10.1007/s10877-016-9942-5>
- 597 Davies, D. J., Su, Z., Clancy, M. T., Lucas, S. J., Dehghani, H., Logan, A., & Belli, A.  
598 (2015). Near-Infrared Spectroscopy in the Monitoring of Adult Traumatic Brain Injury:  
599 A Review. *J Neurotrauma*, *32*(13), 933–941. <https://doi.org/10.1089/neu.2014.3748>
- 600 Davranche, K., Casini, L., Arnal, P. J., Rupp, T., Perrey, S., & Verges, S. (2016). Cognitive  
601 functions and cerebral oxygenation changes during acute and prolonged hypoxic  
602 exposure. *Physiology & Behavior*, *164*(Pt A), 189–197.  
603 <https://doi.org/10.1016/j.physbeh.2016.06.001>
- 604 Dykiert, D., Hall, D., van Gemenen, N., Benson, R., Der, G., Starr, J. M., & Deary, I. J.  
605 (2010). The effects of high altitude on choice reaction time mean and intra-individual  
606 variability: Results of the Edinburgh Altitude Research Expedition of 2008.  
607 *Neuropsychology*, *24*(3), 391–401. <https://doi.org/10.1037/a0018502>
- 608 Earles, J. L., Kersten, A. W., Berlin Mas, B., & Miccio, D. M. (2004). Aging and memory for  
609 self-performed tasks: effects of task difficulty and time pressure. *J Gerontol B Psychol*  
610 *Sci Soc Sci*, *59*(6), P285-93.
- 611 Gallego, J., Perruchet, P., & Camus, J. F. (1991). Assessing attentional control of breathing  
612 by reaction time. *Psychophysiology*, *28*(2), 217–224.
- 613 Hackett, P. H., & Roach, R. C. (2001). High- Altitude Illness. *N Engl J Med*, *345*(2), 107–  
614 114. <https://doi.org/10.1056/NEJM200107123450206>
- 615 Kety, S. S., & Schmidt, C. F. (1946). The effects of active and passive hyperventilation on  
616 cerebral blood flow, cerebral oxygen consumption, cardiac output, and blood pressure of  
617 normal young men. *J Clin Invest*, *25*, 107–119.
- 618 Kety, S. S., & Schmidt, C. F. (1948). The effects of altered arterial tensions of carbon dioxide  
619 and oxygen on cerebral blood flow and cerebral oxygen consumption of normal young  
620 men. *J Clin Invest*, *27*(4), 484. <https://doi.org/10.1172/JCI101995>
- 621 Kwon, Y. H., Kwon, J. W., & Park, J. W. (2013). Changes in brain activation patterns  
622 according to cross-training effect in serial reaction time task: An functional MRI study.  
623 *Neural Regen Res*, *8*(7), 639–646. <https://doi.org/10.3969/j.issn.1673-5374.2013.07.008>
- 624 Laffey, J. G., & Kavanagh, B. P. (2002). Hypocapnia. *N Engl J Med*, *347*(1), 43–53.  
625 <https://doi.org/10.1056/NEJMra012457>
- 626 Lawley, J. S., Macdonald, J. H., Oliver, S. J., & Mullins, P. G. (2017). Unexpected reductions  
627 in regional cerebral perfusion during prolonged hypoxia. *The Journal of Physiology*,  
628 *595*(3), 935–947. <https://doi.org/10.1113/JP272557>
- 629 Lowe, C., & Rabbitt, P. (1998). Test/re-test reliability of the CANTAB and ISPOCD  
630 neuropsychological batteries: theoretical and practical issues. Cambridge  
631 Neuropsychological Test Automated Battery. International Study of Post-Operative  
632 Cognitive Dysfunction. *Neuropsychologia*, *36*(9), 915–923.

- 633 Lucas, S. J. E., Burgess, K. R., Thomas, K. N., Donnelly, J., Peebles, K. C., Lucas, R. A. I.,  
634 ... Ainslie, P. N. (2011). Alterations in cerebral blood flow and cerebrovascular  
635 reactivity during 14 days at 5050 m. *Journal of Physiology*, 589(3), 741–753.  
636 <https://doi.org/10.1113/jphysiol.2010.192534>
- 637 Malatino, L. S., Bellofiore, S., Costa, M. P., Lo Manto, G., Finocchiaro, F., & Di Maria, G.  
638 U. (1992). Cerebral blood flow velocity after hyperventilation-induced vasoconstriction  
639 in hypertensive patients. *Stroke*, 23(12), 1728–1732.  
640 <https://doi.org/10.1161/01.STR.23.12.1728>
- 641 Malle, C., Quinette, P., Laisney, M., Bourrilhon, C., Boissin, J., Desgranges, B., ... Pierard,  
642 C. (2013). Working memory impairment in pilots exposed to acute hypobaric hypoxia.  
643 *Aviat Space Environ Med*, 84(8), 773–779.
- 644 Mardimae, A., Balaban, D. Y., Machina, M. A., Battisti-Charbonney, A., Han, J. S.,  
645 Katznelson, R., ... Duffin, J. (2012). The interaction of carbon dioxide and hypoxia in  
646 the control of cerebral blood flow. *Pflügers Arch*, 464(4), 345–351.  
647 <https://doi.org/10.1007/s00424-012-1148-1>
- 648 Matos Goncalves, M., Pinho, M. S., & Simoes, M. R. (2018). Construct and concurrent  
649 validity of the Cambridge neuropsychological automated tests in Portuguese older adults  
650 without neuropsychiatric diagnoses and with Alzheimer's disease dementia.  
651 *Neuropsychology, Development, and Cognition. Section B, Aging, Neuropsychology and*  
652 *Cognition*, 25(2), 290–317. <https://doi.org/10.1080/13825585.2017.1294651>
- 653 Mutch, W. A. C., Patel, S. R., Shahidi, A. M., Kulasekara, S. I., Fisher, J. A., Duffin, J., &  
654 Hudson, C. (2013). Cerebral Oxygen Saturation: Graded Response to Carbon Dioxide  
655 with Isoxia and Graded Response to Oxygen with Isocapnia. *PLoS ONE*, 8(2), e57881.  
656 <https://doi.org/10.1371/journal.pone.0057881>
- 657 Petrassi, F. A., Hodkinson, P. D., Walters, P., & Gaydos, S. J. (2012). Hypoxic Hypoxia at  
658 Moderate Altitudes: Review of the State of the Science. *Aviat Space Environ Med*, 83,  
659 975–984. <https://doi.org/10.3357/ASEM.3315.2012>
- 660 Pun, M., Hartmann, S. E., Furian, M., Dyck, A. M., Muralt, L., Lichtblau, M., ... Poulin, M.  
661 J. (2018). Effect of Acute, Subacute, and Repeated Exposure to High Altitude (5050 m)  
662 on Psychomotor Vigilance. *Frontiers in Physiology*, 9, 677.  
663 <https://doi.org/10.3389/fphys.2018.00677>
- 664 Robbins, P. A., Swanson, G. D., & Howson, M. G. (1982). A prediction-correction scheme  
665 for forcing alveolar gases along certain time courses. *Journal of Applied Physiology:*  
666 *Respiratory, Environmental and Exercise Physiology*, 52(5), 1353–1357.  
667 <https://doi.org/10.1152/jappl.1982.52.5.1353>
- 668 Saunders, N. L. J., & Summers, M. J. (2010). Attention and working memory deficits in mild  
669 cognitive impairment. *Journal of Clinical and Experimental Neuropsychology*, 32(4),  
670 350–357. <https://doi.org/10.1080/13803390903042379>
- 671 Savourey, G., Launay, J.-C., Besnard, Y., Guinet, A., & Travers, S. (2003). Normo- and  
672 hypobaric hypoxia: are there any physiological differences? *European Journal of*  
673 *Applied Physiology*, 89(2), 122–126. <https://doi.org/10.1007/s00421-002-0789-8>
- 674 Smith, P. J., Need, A. C., Cirulli, E. T., Chiba-Falek, O., & Attix, D. K. (2013). A

- 675 comparison of the Cambridge Automated Neuropsychological Test Battery (CANTAB)  
676 with “traditional” neuropsychological testing instruments. *Journal of Clinical and*  
677 *Experimental Neuropsychology*, 35(3), 319–328.  
678 <https://doi.org/10.1080/13803395.2013.771618>
- 679 Smith, Z. M., Krizay, E., Guo, J., Shin, D. D., Scadeng, M., & Dubowitz, D. J. (2012).  
680 Sustained high-altitude hypoxia increases cerebral oxygen metabolism. *Journal of*  
681 *Applied Physiology*, 114(1), 11–18. <https://doi.org/10.1152/jappphysiol.00703.2012>
- 682 Subudhi, A. W., Fan, J., Evero, O., Bourdillon, N., Kayser, B., Julian, C. G., ... Roach, R. C.  
683 (2014). AltitudeOmics: effect of ascent and acclimatization to 5260 m on regional  
684 cerebral oxygen delivery. *Experimental Physiology*, 99(5), 772–781.  
685 <https://doi.org/10.1113/expphysiol.2013.075184>
- 686 Subudhi, A. W., Miramon, B. R., Granger, M. E., & Roach, R. C. (2009). Frontal and motor  
687 cortex oxygenation during maximal exercise in normoxia and hypoxia. *J Appl Physiol*  
688 (1985), 106(4), 1153–1158. <https://doi.org/10.1152/jappphysiol.91475.2008>
- 689 Szabo, K., Lako, E., Juhasz, T., Rosengarten, B., Csiba, L., & Olah, L. (2011). Hypocapnia  
690 induced vasoconstriction significantly inhibits the neurovascular coupling in humans. *J*  
691 *Neurol Sci*, 309(1–2), 58–62. <https://doi.org/10.1016/j.jns.2011.07.026>
- 692 Turner, C. E., Barker-Collo, S. L., Connell, C. J., & Gant, N. (2015). Acute hypoxic gas  
693 breathing severely impairs cognition and task learning in humans. *Physiol Behav*, 142,  
694 104–110. <https://doi.org/10.1016/j.physbeh.2015.02.006>
- 695 van Asselen, M., Kessels, R. P., Neggers, S. F., Kappelle, L. J., Frijns, C. J., & Postma, A.  
696 (2006). Brain areas involved in spatial working memory. *Neuropsychologia*, 44(7),  
697 1185–1194. <https://doi.org/10.1016/j.neuropsychologia.2005.10.005>
- 698 Van Diest, I., Stegen, K., Van de Woestijne, K. P., Schippers, N., & Van den Bergh, O.  
699 (2000). Hyperventilation and attention: effects of hypocapnia on performance in a stroop  
700 task. *Biol Psychol*, 53(2–3), 233–252.
- 701 Van Dorp, E., Los, M., Dirven, P., Sarton, E., Valk, P., Teppema, L., ... Dahan, A. (2007).  
702 Inspired carbon dioxide during hypoxia: effects on task performance and cerebral  
703 oxygen saturation. *Aviat Space Environ Med*, 78(7), 666–672.
- 704 Verbree, J., Bronzwaer, A.-S. G. T., Ghariq, E., Versluis, M. J., Daemen, M. J. A. P., van  
705 Buchem, M. A., ... van Osch, M. J. P. (2014). Assessment of middle cerebral artery  
706 diameter during hypocapnia and hypercapnia in humans using ultra-high-field MRI.  
707 *Journal of Applied Physiology*, 117(10), 1084–1089.  
708 <https://doi.org/10.1152/jappphysiol.00651.2014>
- 709 Vestergaard, M. B., Lindberg, U., Aachmann-Andersen, N. J., Lisbjerg, K., Christensen, S. J.,  
710 Law, I., ... Larsson, H. B. W. (2015). Acute hypoxia increases the cerebral metabolic  
711 rate – a magnetic resonance imaging study. *Journal of Cerebral Blood Flow &*  
712 *Metabolism*, 36(6), 1046–1058. <https://doi.org/10.1177/0271678X15606460>
- 713 Wang, K., Smith, Z. M., Buxton, R. B., Swenson, E. R., & Dubowitz, D. J. (2015).  
714 Acetazolamide during acute hypoxia improves tissue oxygenation in the human brain.  
715 *Journal of Applied Physiology (Bethesda, Md. : 1985)*, 119(12), 1494–1500.  
716 <https://doi.org/10.1152/jappphysiol.00117.2015>

- 717 Willie, C K., Tzeng, Y. C., Fisher, J. A., & Ainslie, P. N. (2014). Integrative regulation of  
718 human brain blood flow. *Journal of Physiology*, *592*(5), 841–859.  
719 <https://doi.org/10.1113/jphysiol.2013.268953>
- 720 Willie, C K, Colino, F. L., Bailey, D. M., Tzeng, Y. C., Binsted, G., Jones, L. W., ... Ainslie,  
721 P. N. (2011). Utility of transcranial Doppler ultrasound for the integrative assessment of  
722 cerebrovascular function. *J Neurosci Methods*, *196*(2), 221–237.  
723 <https://doi.org/10.1016/j.jneumeth.2011.01.011>
- 724 Wilson, M. H., Edsell, M. E., Davagnanam, I., Hirani, S. P., Martin, D. S., Levett, D. Z., ...  
725 Caudwell Xtreme Everest Research Group. (2011). Cerebral Artery Dilatation Maintains  
726 Cerebral Oxygenation at Extreme Altitude and in Acute Hypoxia—An Ultrasound and  
727 MRI Study. *Journal of Cerebral Blood Flow & Metabolism*, *31*(10), 2019–2029.  
728 <https://doi.org/10.1038/jcbfm.2011.81>
- 729 Yan, X. (2014). Cognitive Impairments at High Altitudes and Adaptation. *High Alt Med Biol*,  
730 *15*(2), 141–145. <https://doi.org/10.1089/ham.2014.1009>

**Absolute Resting Haemodynamic and Gas Values during Isocapnic Euoxic and Experimental Conditions**

	HR (bpm)	MAP (mmHg)	MCAV (cm·s <sup>-1</sup> )	TOI (%)	nTHI (au)	PET <sub>O<sub>2</sub></sub> (mmHg)	PETCO <sub>2</sub> (mmHg)
<b>Isocapnic Euoxic (n=20)</b>							
Isocapnic Hypoxia	67.3 ± 10.8	82.4 ± 15.7	65.4 ± 11.8			98.9 ± 3.9	41.1 ± 2.1
Poikilocapnic Hypoxia	68.1 ± 11.3	87.6 ± 14.7	64.9 ± 12.2			98.8 ± 4.2	41.5 ± 2.0
<b>Subgroup (n=10)</b>							
Isocapnic Hypoxia	60.9 ± 7.2	77.4 ± 10.9	61.6 ± 12.2	74.9 ± 4.9	0.98 ± 0.08	99.6 ± 4.0	40.8 ± 1.7
Poikilocapnic Hypoxia	63.4 ± 8.2	82.3 ± 11.8	60.9 ± 11.0	73.7 ± 3.7	1.00 ± 0.06	100.8 ± 3.8	41.2 ± 2.4
Euoxic Hypocapnia	63.4 ± 8.7	77.4 ± 12.7	61.9 ± 9.9	73.3 ± 3.8	1.00 ± 0.05	97.0 ± 3.3	41.6 ± 1.3
<b>Experimental (n=20)</b>							
Isocapnic Hypoxia	68.9 ± 10.3	90.8 ± 14.0	72.2 ± 11.8 <sup>α**</sup>			43.6 ± 1.7 <sup>**</sup>	41.4 ± 2.2 <sup>α</sup>
Poikilocapnic Hypoxia	71.0 ± 10.6	86.4 ± 10.3	67.3 ± 11.0			42.2 ± 2.9 <sup>**</sup>	39.0 ± 3.2 <sup>**</sup>
<b>Subgroup (n=10)</b>							
Isocapnic Hypoxia	63.9 ± 6.3	87.5 ± 14.4	68.5 ± 10.8 <sup>αβ*</sup>	66.3 ± 4.6 <sup>β**</sup>	1.02 ± 0.09 <sup>β*</sup>	43.4 ± 1.8 <sup>β**</sup>	41.3 ± 2.1 <sup>αβ</sup>
Poikilocapnic Hypoxia	65.1 ± 8.6	84.2 ± 9.9	62.2 ± 9.1	64.7 ± 4.8 <sup>β**</sup>	1.03 ± 0.08 <sup>β</sup>	42.5 ± 3.6 <sup>β**</sup>	37.9 ± 3.5 <sup>β**</sup>
Euoxic Hypocapnia	61.0 ± 9.4	85.6 ± 11.3	51.7 ± 7.6 <sup>**</sup>	68.8 ± 4.1 <sup>**</sup>	0.95 ± 0.05 <sup>**</sup>	97.0 ± 3.6	33.3 ± 1.5 <sup>**</sup>

731 **Table 1.** Absolute resting values for cerebral haemodynamics and end-tidal respiratory gases during isocapnic euoxic baseline and experimental  
732 conditions. Experimental conditions were isocapnic hypoxia (IH), poikilocapnic hypoxia (PH), and euoxic hypocapnia (EH). Data are presented  
733 for the group which completed IH and PH conditions (n = 20), and for the subgroup which completed the additional EH condition (n = 10).  
734 Significance notation represents differences between data pooled across four measured time points during each IE and experimental period. \* p <  
735 0.05 compared to IE. \*\* p < 0.001 compared to IE. α p < 0.05 compared to PH. β p < 0.05 compared to EH. HR, Heart rate; MAP, Mean arterial  
736 pressure; MCAV, Middle cerebral artery velocity; TOI, Total oxygenation index; nTHI, Total haemoglobin index normalised to initial value;  
737 PET<sub>O<sub>2</sub></sub>, End-tidal partial pressure of oxygen; PETCO<sub>2</sub>, End-tidal partial pressure of carbon dioxide Values are Mean ± SD.

**Cognitive Task Performance during Isocapnic Euoxic and Experimental Conditions**

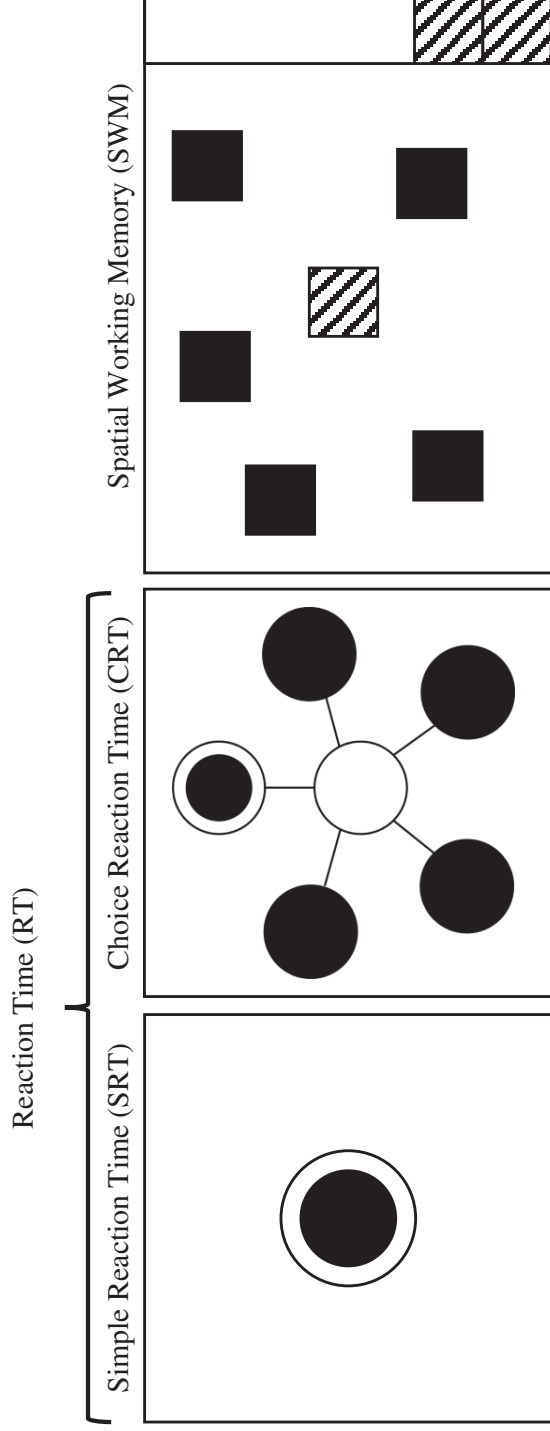
	SRT		CRT		SWM	
	Time (ms)	Error Count	Time (ms)	Error Count	Error Count	Error Count
<b>Isocapnic Euoxic (n=20)</b>						
Isocapnic Hypoxia	573 ± 55	0.3 ± 0.5	590 ± 60	0.8 ± 0.7	7.9 ± 12.8	7.9 ± 12.8
Poikilocapnic Hypoxia	552 ± 52	0.4 ± 0.5	583 ± 74	0.9 ± 1.3	9.5 ± 12.9	9.5 ± 12.9
<b>Subgroup (n=10)</b>						
Isocapnic Hypoxia	550 ± 47	0.3 ± 0.5	579 ± 58	0.8 ± 0.6	8.1 ± 15.9	8.1 ± 15.9
Poikilocapnic Hypoxia	532 ± 46	0.6 ± 0.5	562 ± 72	1.4 ± 1.5	7.3 ± 15.1	7.3 ± 15.1
Euoxic Hypocapnia	544 ± 31	0.5 ± 0.7	588 ± 69	0.6 ± 0.8	5.6 ± 11.8	5.6 ± 11.8
<b>Experimental (n=20)</b>						
Isocapnic Hypoxia	575 ± 54	0.5 ± 0.6	600 ± 75	0.8 ± 0.8	8.1 ± 17.5	8.1 ± 17.5
Poikilocapnic Hypoxia	700 ± 85 <sup>δ**</sup>	0.3 ± 0.4	735 ± 86 <sup>δ**</sup>	0.6 ± 0.8	11.5 ± 13.3	11.5 ± 13.3
<b>Subgroup (n=10)</b>						
Isocapnic Hypoxia	566 ± 51	0.3 ± 0.5	594 ± 70	1.0 ± 0.8	6.4 ± 14.0	6.4 ± 14.0
Poikilocapnic Hypoxia	721 ± 51 <sup>δ**</sup>	0.3 ± 0.5	765 ± 47 <sup>δ**</sup>	0.6 ± 1.0	9.8 ± 17.2	9.8 ± 17.2
Euoxic Hypocapnia	718 ± 55 <sup>δ**</sup>	0.6 ± 0.8	755 ± 34 <sup>δ**</sup>	0.2 ± 0.6	13.0 ± 15.5	13.0 ± 15.5

738

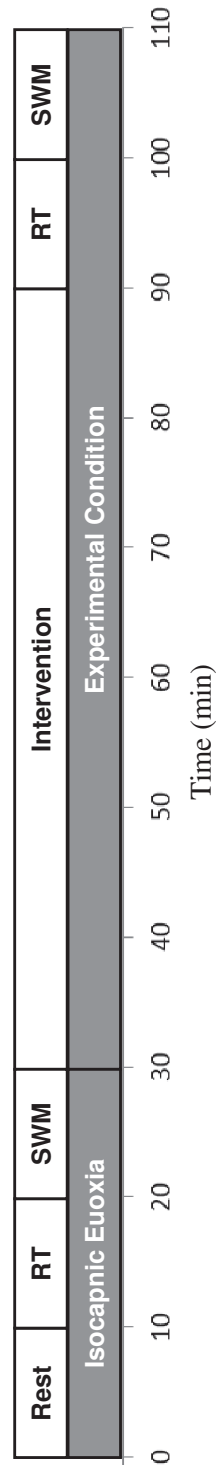
739 **Table 2.** Performance time and error count for simple reaction time (SRT) and five-choice reaction time (CRT) tasks, and error count for spatial  
740 working memory (SWM) task during isocapnic euoxic and experimental conditions. Experimental conditions were isocapnic hypoxia (IH),  
741 poikilocapnic hypoxia (PH) and euoxic hypocapnia (EH). Data have been presented for the group (n=20) which completed the IH and PH  
742 conditions, and for the subgroup (n=10) which completed the additional EH condition. \*\* p < 0.001 compared to IE.  $\delta$  p < 0.001 compared to IH.  
743 Values are Mean ± SD

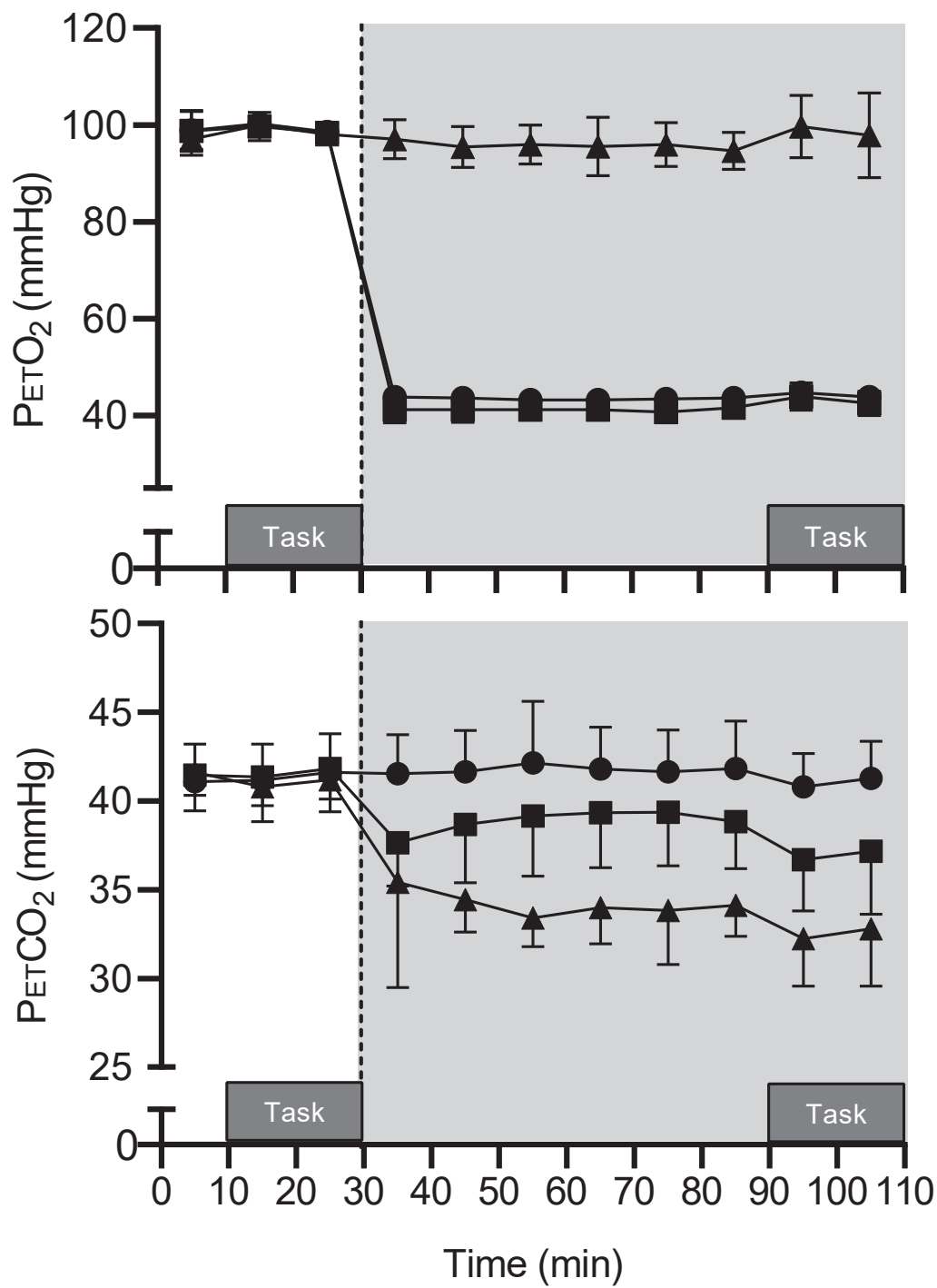


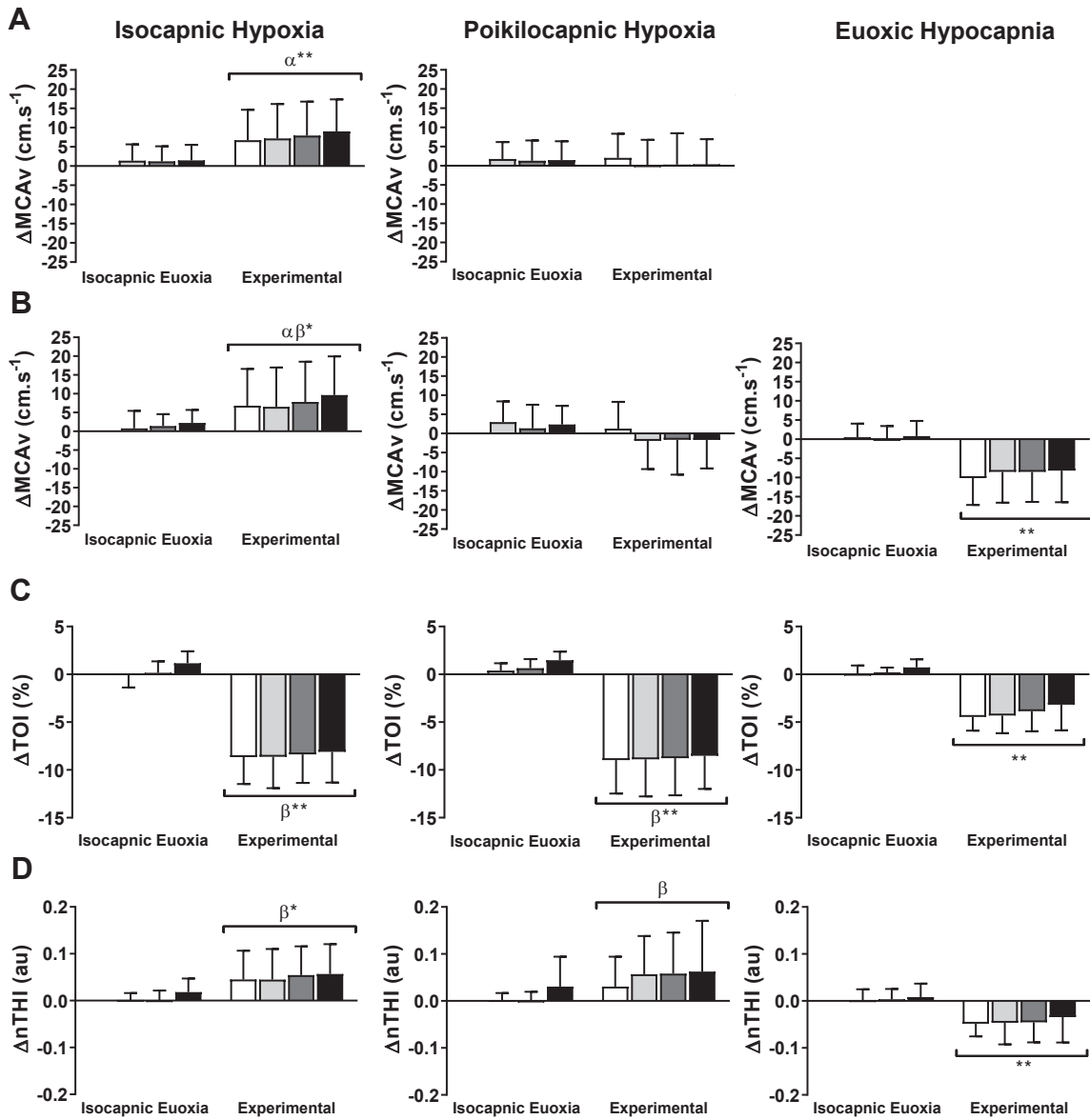
A)



B)







● Isocapnic Hypoxia    ◆ Poikilocapnic Hypoxia    ■ Euoxic Hypocapnia

