Insights Into Cerebral Tissue-Specific Response to Respiratory Challenges at 7T: Evidence for Combined Blood Flow and CO\(_2\)-Mediated Effects

Allen A. Champagne\(^1,2\) and Alex A. Bhogal\(^3\)*

\(^1\) Centre for Neuroscience Studies, Queen's University, Kingston, ON, Canada, \(^2\) School of Medicine, Queen's University, Kingston, ON, Canada, \(^3\) Department of Radiology, University Medical Center Utrecht, Utrecht, Netherlands

Cerebrovascular reactivity (CVR) mapping is finding increasing clinical applications as a non-invasive probe for vascular health. Further analysis extracting temporal delay information from the CVR response provide additional insight that reflect arterial transit time, blood redistribution, and vascular response speed. Untangling these factors can help better understand the (patho)physiology and improve diagnosis/prognosis associated with vascular impairments. Here, we use hypercapnic (HC) and hyperoxic (HO) challenges to gather insight about factors driving temporal delays between gray-matter (GM) and white-matter (WM). Blood Oxygen Level Dependent (BOLD) datasets were acquired at 7T in nine healthy subjects throughout BLOCK- and RAMP-HC paradigms. In a subset of seven participants, a combined HC+HO block, referred as the “BOOST” protocol, was also acquired. Tissue-based differences in Rapid Interpolation at Progressive Time Delays (RIPTiDe) were compared across stimulus to explore dynamic (BLOCK-HC) versus progressive (RAMP-HC) changes in CO\(_2\), as well as the effect of bolus arrival time on CVR delays (BLOCK-HC versus BOOST). While GM delays were similar between the BLOCK- (21.80 ± 10.17 s) and RAMP-HC (24.29 ± 14.64 s), longer WM lag times were observed during the RAMP-HC (42.66 ± 17.79 s), compared to the BLOCK-HC (34.15 ± 10.72 s), suggesting that the progressive stimulus may predispose WM vasculature to longer delays due to the smaller arterial content of CO\(_2\) delivered to WM tissues, which in turn, decreases intravascular CO\(_2\) gradients modulating CO\(_2\) diffusion into WM tissues. This was supported by a maintained ∼10 s offset in GM (11.66 ± 9.54 s) versus WM (21.40 ± 11.17 s) BOOST-delays with respect to the BLOCK-HC, suggesting that the vasoactive effect of CO\(_2\) remains constant and that shortening of BOOST delays was be driven by blood arrival reflected through the non-vasodilatory HO contrast. These findings support that differences in temporal and magnitude aspects of CVR between vascular networks reflect a component of CO\(_2\) sensitivity, in addition to redistribution and steal blood flow effects. Moreover, these results emphasize that the addition of a BOOST paradigm may provide clinical
INTRODUCTION

Cerebrovascular tone is constantly being modulated to ensure adequate supply of oxygen and glucose to brain tissues (Paulson et al., 1990; Iadecola and Nedergaard, 2007). Control arterioles that branch from larger feeding arteries respond to local changes in metabolism, pH, or arterial CO$_2$ partial pressure (P$_a$CO$_2$), work to regulate cerebral blood flow (CBF), ensuring proper delivery of nutrients and effective waste removal. This sensitive process, whereby dynamic changes in vascular tone modulate regional CBF in response to vasodilatory stimuli is defined as cerebrovascular reactivity [CVR; (Fisher et al., 2018; Liu et al., 2018)]. In recent years, CVR mapping has emerged as a robust clinical tool to understand the vascular physiology underlying conditions like stroke (Geranmayeh et al., 2015), moyamoya disease (Conklin et al., 2010), arterial stenosis (Mandell et al., 2008a), and traumatic brain injury (Len et al., 2011; Ellis et al., 2016; Champagne et al., 2020), as well as the hemodynamic processes associated with normal aging of cerebral tissues (De Vis et al., 2015; Bhogal et al., 2016).

Moving beyond amplitude-based assessments of vascular properties (i.e., CVR) advanced post-processing analyzes have emerged to characterize temporal markers associated with the CVR response (Duffin et al., 2015; Poublanc et al., 2015; Donahue et al., 2016; van Niftrik et al., 2017; Champagne et al., 2019). These markers can provide quantitative profiles that aid in the explanation of differences between vasodilatory response across cortical and sub-cortical tissues (Donahue et al., 2014; Thomas et al., 2014; Champagne et al., 2019) as well as pathological tissues with impaired or temporally altered hemodynamics due to collateralization (Donahue et al., 2016). The general consensus is that temporal characteristics of the vascular response reflect a combination of factors including arterial transit time, blood redistribution, and vascular response speed, which may be partially separated using a combination of hypercapnic (HC) respiratory challenges to stress the cerebral vasculature and hyperoxic (HO) respiratory challenges that have previously been implemented to act as endogenous contrast agents via O$_2$-mediated changes in deoxyhemoglobin (Blockley et al., 2013; Champagne et al., 2019).

Through examinations of cerebrovascular architecture, it is known that cortical and deep gray-matter (GM) tissues have high arteriolar density (Moody et al., 1990), which are fed by larger vessels branching off major cerebral arteries (i.e., pial, lenticulostriate, and choroidal). During HC, arteriolar smooth-vessel mediated dilation decreases peripheral resistance in cerebral arteries leading to an increase in CBF through capillary beds in accordance with Poiseuille’s law (McDonald, 1974; Edvinsson et al., 2002). The bulk of these arterial resistance changes are in reaction to rising extravascular P$_a$CO$_2$ that, in turn, drives changes in blood pH and local vasodilatory mechanisms (Yoon et al., 2000; Tripp et al., 2001; Chesler et al., 2008). During MR imaging of HC, fractional changes in BOLD signal arise from the relationship between regional increases in blood flow and subsequent decreases in deoxy-hemoglobin concentration ([d-Hb]), which lengths T$_2^*$ relaxation (Ogawa et al., 1990, 1993). Assuming this coupling between CBF and [d-Hb] remains constant, differences in local CO$_2$ accumulation, sensitivity and cross-membrane diffusion rate (Thomas et al., 2014), as well as the possible redistribution (or “vascular-steal”) effects between vascular networks (Conklin et al., 2010; Fierstra et al., 2010; Bhogal et al., 2015), may all bias CVR measurements derived using standard methods, emphasizing the need to include additional temporal marker for the cerebrovascular response.

While HC modulates the BOLD signal via changes in perfusion, the BOLD contrast can also be manipulated when combined with non-vasoactive HO breathing challenges (Blockley et al., 2013). As the arterial partial pressure of oxygen (P$_a$O$_2$) increases, complete hemoglobin saturation at the lungs drives the dissolution of abundant O$_2$ into the blood plasma. This O$_2$-loaded blood traverses the cardio-vascular network until it reaches the capillary beds where it diffuses toward cerebral tissues to meet metabolic demand. In terms of the BOLD contrast mechanisms, the increase in O$_2$ supply results in a reduced unloading of O$_2$ from arterial hemoglobin and a concomitant decrease in venous de-oxyhemoglobin content; this lengthens the blood T$_2^*$ relaxation time, creating a similar BOLD signal response as what is observed during HC. This O$_2$ based mechanism has been proposed as a means through which arrival time (Champagne et al., 2019) and cerebral blood volume (CBV) (Lu et al., 2003) can be assessed and assumes limited vasostenotonic and minor reduction in venous CBV (Bulte et al., 2007; Mark and Pike, 2012; Xu et al., 2012). Irrespective of potential vaso-constrictive effects, the bolus of highly saturated blood formed during HO breathing can act as a non-invasive endogenous contrast agent for estimation of bolus arrival time (Blockley et al., 2013; Liu et al., 2017; Champagne et al., 2019) since only minor reductions in blood velocity would be expected. Consequently, HO may be used to calibrate HC-derived CVR delays, and untangle physiological factors that contribute to the (patho)physiological mechanisms associated with diseases causing vascular impairments.

In MR-based methods that probe CVR using a gas delivery system (Slessarev et al., 2007; Mark et al., 2010; Fisher, 2016), HC is typically induced via inhalation of CO$_2$ administered using a block or ramp respiratory challenge [see Figure 4 in Liu et al. (2018)]. Block-based HC is a stimulus where the end-tidal CO$_2$ (PETCO$_2$) is rapidly increased from baseline in a boxcar function (typically +5–10 mmHg), which is assumed to evoke a dynamic CVR response (van der Zande et al., 2005). In contrast, the
RAMP-HC stimulus involves a gradual increase in the $P_{ET}CO_2$ which is assumed to allow for more progressive changes in local $P_aCO_2$ (Ringlestein et al., 1988; Claassen et al., 2006; Battist-Charbonney et al., 2011; Bhogal et al., 2014b). Although the step design is commonly used to characterize temporal CVR delays, no studies to date has yet to explore tissue-based differences in delays from the vascular response induced during a ramp design, and how those may differ from CVR delays derived from block designs. Based on the assumption that local $CO_2$ levels are increased progressively during the ramp, it may be hypothesized that the slower arteriolar reactivity in response to decreasing pH allows for less blood flow redistribution and steal effects between vascular territories and tissues, that in turn, lead to shorter CVR delays. Additionally, given that HO-based BOLD mapping can be leveraged to estimate bolus arrival time, it may be hypothesized that a combined boxcar HC and HO respiratory stimulus can provide additional insight with respect to possible redistribution effects within local vessels, in the event that the region-specific lag times remain relatively increased compared to neighboring vascular territories.

In this study, we integrate HC- and HO-based respiratory challenges to gather insight about factors driving tissues-based differences in temporal delays of the CVR response. High spatial-resolution BOLD datasets were acquired at 7T using both BLOCK- and RAMP-HC paradigms to study the potential differences in CVR delays between dynamic and progressive local changes in $CO_2$. Finally, a novel protocol using a combined HC and HO block, referred as the “BOOST” protocol, was used to study the effect of bolus arrival time on tissue-based CVR delays, in contrast to $CO_2$ sensitivity.

MATERIALS AND METHODS

Subjects
We retrospectively surveyed our seven Tesla subject database to identify subjects in which both RAMP- and BLOCK-HC designs were acquired using the same MR imaging readout. We were able to identify a total of nine subjects. In a subset of seven of these subjects, an additional paradigm was identified in which a HO–HC block (herein termed “BLOCK BOOST HC+HO”) was also administered. Age and gender information for the main (herein termed SAMPLE.1) and sub-group (herein termed SAMPLE.2) are provided in Table 1.

Respiratory Paradigms
Respiratory challenges were delivered using a 3rd generation RespirAct™ (Thornhill Research Inc., Toronto, ON, Canada) system in combination with a rebreathing face mask. The mask was taped to ensure an air-tight seal using Tegaderm transparent dressing (3M, St. Paul, MN, United States). For both groups, the BLOCK-HC protocol consisted of a 90s increase in hypercapnia, preceded, and followed by a pre- and post-baseline period, respectively (Figure 1A). In the case of the combined BLOCK BOOST HC+HO experiment, a simultaneous increase in arterial $O_2$ was implemented (Figure 1A). To ensure sharp transitions in arterial $O_2$, the level of the $O_2$ increase was limited such that the gas delivery system could evoke arterial changes within several breaths in line with the transitions possible when increasing arterial $CO_2$ (Figure 1A). The progressive protocol (i.e., RAMP-HC) consisted of a baseline period followed by a 60 s hypocapnic period and a subsequent 5-min period of progressively increasing hypercapnia, ending with a return to baseline (Figure 1A). Ramped $CO_2$ protocols are well suited to determine the precise vascular reserve capacity in a particular region. For example, impaired CVR will manifest as an early plateau in the BOLD-CVR response as vessels exhaust their dilatory reserve capacity. The progressive nature of the ramped stimulus means that the subject only experiences the high arterial $CO_2$ levels required to elucidate mild impairments toward the end of the paradigm. In contrast, protocols based on BLOCK stimuli are suited to reveal dynamic aspects of the BOLD-CVR response and are imprecise in terms of revealing the dose-response profile of the $CO_2$ stimulus. For this reason, CVR information can be obtained using a lower stimulus magnitude that spares the subject from prolonged high-level exposure that can potentially cause discomfort. With the exception of one subject, all three paradigms were acquired on the same day during the same scan session. The baseline and achieved end-tidal $CO_2$ and $O_2$ values across all paradigms used in our study are reported in Table 1. Typical end-tidal $CO_2$ and $O_2$ traces along with corresponding GM and WM ROI responses are shown for examples of each paradigm in Supplementary Figure 1.

Magnetic Resonance Imaging
This study was approved by the medical research ethics committee of University Medical Center Utrecht (UMCU) and written informed consent was obtained from all subjects. The experiments were performed according to the guidelines and regulations of the WMO (Wet Medisch Wetenschappelijk
FIGURE 1 | Summary schematic of the respiratory paradigms and imaging outputs. Sample data showing the RespirAct™ (Toronto, ON, Canada) end-tidal trace (A) for CO₂ (P\(_{\text{ET}}\)CO₂, mmHg; orange line and purple dot) and O₂ (P\(_{\text{ET}}\)O₂, mmHg; blue line and red dot) during the hypercapnic (HC) and hyperoxic (HO) gas challenges used in this study: BLOCK HC only (top), BLOCK BOOST HC+HO (middle) and RAMP HC only (bottom). The Blood Oxygen Level Dependent (BOLD) signal acquired simultaneously (B) was denoised using a Bayesian wavelet approach on a voxel-by-voxel basis and then extracted from the gray matter (C) to compute the optimized BOLD regressor using the Rapid Interpolation at Progressive Time Delays (RIPTiDe) method. The final BOLD regressor was then cross correlated against the original denoised BOLD signal (D) at each voxel to compute voxelwise lag maps (seconds) (E). This was repeated for each breathing challenges shown in (A), for each subject.

Onderzoek). Subjects were scanned on a Philips 7 Tesla MRI system using a dual channel transmit coil in combination with a 32-channel receive coil. Image based (3rd order) shimming was performed. Respiratory challenges were administered throughout a multi-slice single-shot GE-EPI BOLD scan (flip angle: 90°, TR/TE 3000/25 ms, EPI/SENSE factor 47/3, reconstructed resolution: 1.5 mm × 1.5 mm, slice thickness: 1.6 mm, no slice gap, FOV: 217.6 mm × 192 mm, slices: 43, respiratory paradigm dependent scan duration: 507s for RAMP-HC and approx. 330s for BLOCK-HC/BOOST HC+HO).

Computation of Vascular Reactivity and Temporal Lags

Post-processing of the BOLD data consisted of temporal re-alignment [FSL:MCFLIRT; (Jenkinson et al., 2012)], segmentation [FSL:FAST; (Zhang et al., 2001)] and voxel-wise temporal de-noising using a Bayesian wavelet based approach (Figure 1B; symlet four wavelet, two levels, hard co-efficient threshold with level dependent noise estimation). End-tidal CO₂ and O₂ traces were resampled to the TR of the BOLD acquisition and aligned to the BOLD data based on the maximum correlation between the breathing trace and the individual mean GM signal. BOLD data and respiratory traces were then interpolated to a temporal resolution of 3000/8 ms (i.e., 8× oversampled) to account for temporal delays between slice acquisitions. The interpolated P\(_{\text{ET}}\)CO₂ traces were used as the initial probe to generate and optimized BOLD signal regressor for a correlation-based temporal lag analysis [RIPTiDe; (Frederick et al., 2012; Tong and Frederick, 2012); Figure 1C]. Based on voxel-wise lag estimates, individual lag maps were generated for the BLOCK-HC, RAMP-HC, and BLOCK BOOST HC+HO respiratory paradigms (Figure 1D). P\(_{\text{ET}}\)CO₂ traces as well as lag-adjusted traces were then used to generate CVR (Δ%BOLD/mmHg) and lag-adjusted CVR.
Comparisons of Vascular Reactivity and Temporal Delays Across Tissues and Paradigms

Segmented tissue-based probability maps were binarized from the 1-mm MINI152 atlas using a voxel-based threshold set to 50 and 90%, for the GM and WM, respectively. The tissue-specific masks were applied to the group-averaged CVR and lag maps to extract the parameter distribution for each respiratory design, along with mean and standard deviation (MATLAB, version 2019a, The Mathworks, MA, United States). This procedure was repeated for both SAMPLE.1 and SAMPLE.2, in order to assess the repeatability of the CVR and delay measurements from the BLOCK- and RAMP- HC. Tissue-based histograms for each average map were also assessed to look at the distribution of the CVR lags, between GM and WM.

RESULTS

End-Tidal Measurements

All end-tidal measurements for each respiratory design are reported in Table 1. On average, participants were exposed to an 8 ± 1 mmHg increase in PETCO2 during the BLOCK- HC, while maintaining changes in PETCO2 fluctuations well below physiological threshold. Similar increases in PETCO2 were obtained during the BLOCK-BOOST (SAMPLE.2), with concurrent increases in PETO2 averaged to 159 ± 44 mmHg, across subjects. During the ramp, larger increases in PETCO2 were obtained, ranging between [20.8–22] ± 6 mmHg, with minimal changes in PETO2.

Cerebrovascular Reactivity and Temporal Delays

All parameters described below are summarized in Table 2. Average CVR measurements for both GM and WM tissues were in agreement between the BLOCK- and RAMP- HC paradigms. In comparison, CVR measurements were higher during the BLOCK-BOOST, although relative differences between the GM and WM were maintained. Qualitatively, similar observations can also be made based on whole brain assessment of lag-corrected CVR (Figure 3).

While GM delays were similar between the BLOCK- and RAMP- HC (within ±2 s), longer WM lag times were observed during the RAMP- HC, when compared to the BLOCK- HC design (Table 2 and Figure 4). This was repeatable across both samples studied (Figure 4). In comparison to both HC designs, shorter GM and WM delays were observed during the BLOCK-BOOST HC+HO protocol, although the relative ~10 s offset in the GM versus WM lag times was maintained. The r² fitting coefficient was similar for the BLOCK- HC and BOOST, within both tissues (Table 2). Greater fitting estimates were observed in the RAMP- HC, compare the block designs, in both the GM and WM masks (Table 2).

DISCUSSION

In this study, we conducted a comprehensive analysis of the response to respiratory gas challenges in GM and WM tissues at 7T, as a way to advance our understanding of the effects that drive temporal delays in the BOLD-CVR response of healthy tissues. Findings from this study were three-fold: (1) In comparison to WM delays during the BLOCK- HC, longer WM delays were observed during the RAMP- HC suggesting that the progressive nature of the ramp stimulus may predispose the WM vasculature to a slower CVR response. We propose that this is a result of the delayed increase in the local intravascular CO2 gradient between blood and tissue compartments. This delayed gradient reduced the driving force facilitating the diffusion of CO2 into the tissues, which in turn, delayed the vasodilatory response. (2) The addition of the HO step within the HC boxcar design for the BLOCK-BOOST HC+HO protocol induced a global decrease in GM and WM delays despite maintaining a ~10 s offset between the tissues, when compared to BLOCK- HC. We postulate that the vasoactive effect of CO2 within tissues remained constant and that the shortening of the delays during BOOST may have been driven by blood arrival effects (i.e., non-vasoactive) arising from the endogenous O2 contrast agent. (3) Comparable magnitude for lag-corrected CVR measurements were reported between the STEP- HC and RAMP- HC protocol, emphasizing that the step design is an appropriate tool to assess CVR markers, despite previous literature suggesting that a ramp-like stimulus may better model the sigmoidal relationship between changes in PETCO2 and CBF, driving BOLD changes.

Table 2 | Gray- and white-matter average parameters for each stimulus.

| Stimulus       | Parameters | GM         | WM         |
|----------------|------------|------------|------------|
| BLOCK HC (N = 9) | CVR (%BOLD/mmHg) | 0.38 ± 0.22 | 0.17 ± 0.11 |
|                | r²         | 0.49 ± 0.12 | 0.39 ± 0.09 |
| RAMP HC (N = 9) | CVR (%BOLD/mmHg) | 0.38 ± 0.22 | 0.18 ± 0.10 |
|                | r²         | 0.54 ± 0.15 | 0.42 ± 0.12 |
| BLOCK BOOST HC+HO (N = 7) | CVR (%BOLD/mmHg) | 0.47 ± 0.28 | 0.21 ± 0.16 |
|                | Lag (seconds) | 11.66 ± 9.54 | 21.40 ± 11.17 |

Values are in mean ± standard deviation.
FIGURE 2 | Corrected cerebrovascular reactivity mapping based on RIPTiDe lag times. Sample data showing BOLD EPI axial slices from the BLOCK-HC challenge in a single subject (A) used to compute the RIPTiDe lag maps (B). The slice number is shown in green. In (C), the matching voxelwise correlation coefficient (r) map (axial, 20) shows the fit between the BOLD signal and optimized RIPTiDe regressor. The original and lag-corrected cerebrovascular reactivity (CVR) maps are shown in (D) and (E), respectively, with a specific look at deeper gray- and white-matter structures, to reflect the improvement in CVR following the lag time correction. Note that cerebrospinal fluid was removed from all maps to improve visual representation of the data. BOLD, Blood Oxygen Level Dependent, HC, hypercapnia, RIPTiDe, Rapid Interpolation at Progressive Time Delays.

in signal (Bhogal et al., 2014a). Altogether, these findings support the hypothesis that differences in temporal components of CVR between vascular networks reflect the culminative effect of CO$_2$ sensitivity (and/or CO$_2$ diffusion rate) in local vessels, in addition to blood flow redistribution and steal effects, as previously described. Moreover, these results suggest that the addition of a BLOCK-BOOST HC+HO paradigm within clinical settings can provide insights into whether diseases causing changes in CVR do so by way of severe blood flow redistribution (which would increase blood arrival time and inflate BLOCK-BOOST HC+HO delays), or alterations in vascular properties within the vessels that could impair CO$_2$ diffusion across WM tissues (i.e., atherosclerosis, amyloid angiopathy, aging-related fibrosis or stiffening of vessels; this would significantly increase the marginal gap in relative delay estimates between BLOCK-HC and BLOCK-BOOST HC+HO).

The GM and WM CVR values reported in this study are in line with previous studies looking at HC designs (Bhogal et al., 2014b, 2015) at higher spatial resolution. That said, the WM CVR values are higher than ones reported using 3T imaging (Thomas et al., 2014; Sam et al., 2016), likely due to a combination of factors including the higher BOLD CNR from 7T imaging, as well as the incorporation of temporal delay correction within the CVR computation, which has been shown to improve CVR estimates in healthy controls (Duffin et al., 2015; Poublanc et al., 2015; Donahue et al., 2016; van Niftrik et al., 2017; Champagne et al., 2019). In this study, lag-corrected WM CVR was found to be lower in the WM, across all breathing paradigms. This is consistent with existing literature (Brian, 1998; Rostrup et al., 2000; Mandell et al., 2008b; Thomas et al., 2014) suggesting that despite temporal corrections for delays in the vascular response, WM tissue may have a lower vasodilatory capacity, at least in part, due to the longer time for extravascular CO$_2$ levels to build within the local vasculature, in response to global increases in arterial CO$_2$ content. As originally proposed in Thomas et al. (2014) (see Figure 3), the delayed intravascular
FIGURE 3 | Average lag-corrected cerebrovascular reactivity maps for each respiratory challenge. (A) The anatomical axial reference slices for the MNI template are shown with the slice number in green. The matching sagittal frame showing the referenced frames is displayed on the left. The group-averaged lag-corrected cerebrovascular reactivity (CVR) maps are displayed for each respiratory challenge in (B–D), representing the BLOCK-HC, BLOCK BOOST HC+HO, and RAMP-HC, respectively. A schematic of each respiratory design is shown on the left with the targeted end-tidal traces for CO$_2$ (yellow) and O$_2$ (cyan blue), as a reference.

build-up in CO$_2$ may be a function of the lower CBF within the WM (Duvernoy et al., 1981), such that, changes in arterial content of CO$_2$ brought to the WM tissues per unit of time are comparatively lower than those for GM. This in turn increases the timespan required within which the intravascular-tissue CO$_2$ gradient can drive trans-membrane diffusion of CO$_2$ toward tissues, and modulate the local increases in extravascular CO$_2$ (which mediates the majority of the HC-induced vasodilation).

This theory is further supported by our observation of longer delays in the WM during the RAMP-HC respiratory paradigm, in comparison to the BLOCK-HC. During a step HC paradigm, the large and rapid increase in arterial CO$_2$ sets up a strong CO$_2$ gradient which remains high throughout the duration of the boxcar stimulus. This effect rapidly saturates GM vessels (due to early arrival time of GM blood) and continues to flow through penetrating arteries toward downstream WM vasculature. This large increase in intravascular CO$_2$ drives the concentration gradient along which CO$_2$ will diffuse to induce the vasodilatory response. In other words, a high gradient leads to a faster and stronger dilatory response whereas a low gradient may induce temporal delays irrespective of blood arrival time; particularly in regions having low overall CBV such as the periventricular WM. The rapid and dynamic nature of the step also provides a setting for physiological steal mechanisms and redistribution of blood flow effects (Sobczyk et al., 2014; Bhogal et al., 2014a; Poublanc et al., 2015; Champagne et al., 2019), by which hyper-sensitive areas may respond quicker to rising levels of arterial CO$_2$, and inflate temporal delays in regionally closely bound vascular territories. This may be especially significant for pathological tissues that show impaired or temporally altered hemodynamic properties due to vascular collateralization (Donahue et al., 2016).

In comparison to the BLOCK-HC, however, the RAMP-HC is assumed to be a progressive stimulus during which the vascular response has sufficient time to equilibrate with changing levels of arteriol CO$_2$ content (and the corresponding
Champagne and Bhogal  Tissue-Based CVR Delays at 7T

FIGURE 4 | Summary of the tissue-based distribution of lag parameters for each respiratory design. (A) The anatomical axial slices for the MNI template providing a reference for the images presented. (B–F) The group-averaged lag maps (seconds) are displayed for each respiratory challenges, sub-divided based on the sample used to compute the mean image (SAMPLE.1, N = 9, top, B–C; SAMPLE.2, N = 7, bottom, D–F). The cumulative percent frequency (normalized to 100%) for the distribution of lag (seconds) is shown for each tissue which was extracted using the gray- (black) and white- (gray) matter mask displayed in (B), bottom right corner. A dotted red line was added to each histogram (B–F) at 40 s, for reference and comparison.

blood-tissue CO$_2$ gradient). Previously this was thought to lead to a more balanced response across brain regions through minimization of the dynamic redistribution (or steal) effects. This mechanisms may explain why GM delays during the RAMP were slightly higher than during the boxcar design (even though this difference was small; within ±2 s), as the slowly rising ramp stimulus allows for the vasculature to respond progressively in healthy tissues, in contrast to the dynamic STEP design which induces a rapid response in the GM vessels. In other words, healthy GM tissues may cope appropriately to rapid changes in arterial CO$_2$ associated with the BLOCK-HC, resulting in relatively shorter response time (and thus, CVR delays). This warrants further exploration in clinical populations where blood flow redistribution effects are much more likely (i.e., Moyamoya disease (Conklin et al., 2010)).

Contrary to our original hypothesis, our results showed that WM CVR delays were longer during the RAMP-HC, in comparison to the BLOCK-HC. We postulate that this occurred as a result of slowly increasing intravascular CO$_2$ associated with the ramp stimulus, paired with limited blood flow to the WM. Together, these effects organically delayed the build-up of higher CO$_2$ gradients within WM tissues, leading to an increase in the time required to evoke the expected vascular response in the WM. This is further exaggerated by additional steal effect from GM regions that are more sensitive due to higher vascular density
(and therefore more CO$_2$ diffusion even considering lower CO$_2$
gradients). The response in WM increases notably toward the end
of the stimulus paradigm, once the ramp reaches higher P$_{ET}$CO$_2$
targets (Figure 1A) that setup stronger CO$_2$ driving gradients.
These findings support the original hypothesis put forward in
Thomas et al. (2014) providing an explanation for the higher
CVR delays in WM within healthy brain tissues, which again, may
be exacerbated in settings where regional blood flow and/or CO$_2$
sensitivity is disturbed.

Building on the findings from the comparison of the BLOCK-
and RAMP-HC, the addition of the HO step during the
HC challenge, using the BLOCK-BOOST HC+HO protocol,
resulted in an overall decrease in the magnitude of delays for
both the GM and WM. Despite those changes, however, the
relative relationship between GM and WM was maintained
during the BOOST protocol, showing a $\sim$10 s offset in delays
between the two cerebral tissues. This supports the findings
described above in that despite correction for arrival time of
the stimulus into local tissues (signaled via the non-vasodilatory
contrast O$_2$ agent), vasodilatory response delays in the WM
were consistently higher than in the GM, suggesting that this
phenomenon may result from slower CO$_2$ gradient build-up
needed to drive diffusion into tissues. In other words, by using
the BOOST protocol to compute CVR delays in clinical settings,
in comparison to the BLOCK-HC delays, a more in-depth
interpretation temporal response differences is possible, which
teaches effects related to the arrival time of the stimulus (where
the magnitude of delays for BLOCK-HC and BOOST would
be similar) versus local impairments in CO$_2$ sensitivity within
WM tissues (which would inflate the $\sim$10 s offset gap between
the GM and WM characterized between the two methods). The
vasoactive mechanisms of CO$_2$ versus O$_2$ may differ and it is
conceivable that their interplay could influence lag estimates.
The elevated CVR resulting from the BOOST HC+HO stimulus
when compared to the BLOCK-HC suggests that either the
vasodilatory effects of the CO$_2$ overwhelmed any constrictive
influence of $O_2$, or the effect of increased $S_2O_2$ increased
the BOLD signal beyond any HO mediated flow reductions.
However, considering the relatively small $O_2$ change applied
in our experiments, we don’t expect appreciable constriction.
Finally, the HO-induced BOLD signal is CBV-weighted, rather
than reflecting CVR. This means that taken alone, the
BOOST HC+HO paradigm may overestimate CVR since the
HO-induced signal contribution is not explicitly accounted
for. Therefore, although the BOOST HC+HO paradigm
may provide additional temporal information about possible
pathophysiological mechanisms, a standard CO$_2$ challenge is
still required for accurate CVR magnitude measurements.
Disentangling respective components of the BOOST HC+HO
response remains interesting for future work.

Beyond the quantitative comparison of delays across the GM
and WM, results from this study show that the distribution of
CVR values across respiratory paradigm were repeatable,
even after adding subjects from SAMPLE.1 to SAMPLE.2. This
emphasizes that voxel-based CVR measurements are consistent
within the brain of healthy controls, allowing for the clinical use
of such biomarker as a tool to provide reference-based voxelwise
analysis of vascular impairments in patients (Sobczyk et al., 2015).
Similarly, the consistency of the CVR delay distribution across
the samples, between respiratory designs and within the GM and
WM, supports that temporal analyses of the CVR response may
too be used as a clinical tool to understand pathophysiological
mechanisms associated with vascular diseases of the brain
(Donahue et al., 2016; Juttukonda and Donahue, 2019).

Despite the novelty of the findings presented in this study,
some limitations must be acknowledged and recognized as
opportunities for future studies. First, the study employed a
retroactive method to analyze datasets that were scanned as
part of branching research studies. This limited our ability to
synchronize and put together complete datasets for all subjects
used in the analysis. Moving forward, a greater cross-sectional
research design may be employed to further assess the use
of the BLOCK-BOOST HC+HO protocol, and its variability
across a larger sample size. Furthermore, only healthy subjects
participated, limiting the external validity of these findings
and preventing our ability to predict whether possible changes
in arrival time, or CO$_2$ sensitivity, as a result of vascular
impairments, may be significant enough to be detected using
the proposed methods. Future work should therefore consider
implementing these analyses in clinical population with known
vascular diseases, in order to assess the potential utility of such
combined approach. Specifically, clinical studies may consider
studying the effect of pathological disease mechanisms on the
local diffusion rate of CO$_2$ into tissues, which could be tested
using a combination of the BLOCK-HC and BLOCK BOOST
HC+HO. It may be hypothesized that local damages to the
endothelium may manifest as increases in the discrepancy
between the GM and WM CVR lag times observed under
each respiratory challenge, while blood arrival remains relatively
unchanged (and thus delays would still be shorter under BLOCK
BOOST HC+HO). Finally, the magnitude of the delivered
BLOCK-HC change in P$_{ET}$CO$_2$ was smaller than the maximum
peak change in P$_{ET}$CO$_2$ reached during the RAMP-HC, which
may impact the values extracted for delays across tissues, given
that the greater stress on local vasculature could promote
a micro-environment within which blood flow redistribution
effects are more apparent across regions with differences in
hemodynamic capacity. Thus, future work may also consider
examining the effect of changing the magnitude of the step
stimulus and exploring whether larger CO$_2$ gradient changes
the voxelwise lag maps.

CONCLUSION

As highlighted in this study, tissue-based differences in CVR
and temporal markers for the response to hypercapnia are
governed by a number of factors that reflects a compounded
effect dependent on arterial arrival time, CO$_2$ sensitivity and
CO$_2$ diffusion rate, blood flow redistribution, and steal effects,
and vascular response speed. Here, we provide evidence showing
that differences in the temporal components of CVR may be
influenced by intravascular CO$_2$ gradients in local vessels, which,
in addition to membrane permeability, and blood flow effects,
determines the rate of diffusion within local tissues that then drives the vasodilatory response. The proposed mechanisms presented in this study, in support of Thomas et al. (2014), may provide a partial explanation for the slower WM response to hypercapnia in comparison to GM, in healthy tissues. Finally, the results presented suggest that the implementation of a BLOCK BOOST HC+HO respiratory challenge may provide additional insight about possible pathophysiological mechanisms underlying vascular diseases based on whether they are driven by severe blood flow redistribution, or alterations in vascular properties within the vessels that would affect trans-membrane CO₂ diffusion into tissues. Moving forward, the combination of a BLOCK-HC and BLOCK-BOOST respiratory paradigms may be used to untangle these factors driving CVR and the speed of response, as a tool to help to improve the diagnosis, prognosis and management of patients with vascular brain diseases.

**DATA AVAILABILITY STATEMENT**

The raw data supporting the conclusions of this article will be made available by the authors, without undue reservation.

**ETHICS STATEMENT**

The studies involving human participants were reviewed and approved by WMO (Wet Medisch Wetenschappelijk Onderzoek). The patients/participants provided their written informed consent to participate in this study.

**REFERENCES**

Battisti-Charbonney, A., Fisher, J., and Duffin, J. (2011). The cerebrovascular response to carbon dioxide in humans. *J. Physiol.* 589, 3039–3048. doi: 10.1113/jphysiol.2011.206052

Bhogal, A. A., De Vis, J. B., Siero, J. C. W., Petersen, E. T., Luijten, P. R., and Hendrikse, J. (2016). The BOLD cerebrovascular reactivity response to progressive hypercapnia in young and elderly. *Neuroimage* 139, 94–102. doi: 10.1016/j.neuroimage.2016.06.010

Bhogal, A. A., Philippens, M. E. P., Siero, J. C. W., Fisher, J. A., Petersen, E. T., Luijten, P. R., et al. (2015). Examining the regional and cerebral depth-dependent BOLD cerebrovascular reactivity response at 7T. *Neuroimage* 114, 239–248. doi: 10.1016/j.neuroimage.2015.04.014

Bhogal, A. A., Siero, J. C. W., Fisher, J. A., Froeling, M., Luijten, P., Philippens, M., et al. (2014a). Investigating the non-linearity of the BOLD cerebrovascular reactivity response to targeted hypo/hypercapnia at 7T. *Neuroimage* 98, 296–305. doi: 10.1016/j.neuroimage.2014.05.006

Bhogal, A. A., Luijten, P. R., Froeling, M., Philippens, M., et al. (2014b). *Neuroimage* investigating the non-linearity of the BOLD cerebrovascular reactivity response to targeted hypo/hypercapnia at 7 T. *Neuroimage* 98, 296–305. doi: 10.1016/j.neuroimage.2014.05.006

Blockley, N. P., Griffith, V. E. M., Germuska, M. A., Bulte, D. P., and Buxton, R. B. (2013). An analysis of the use of hyperoxia for measuring venous cerebral blood volume: comparison of the existing method with a new analysis approach. *Neuroimage* 72, 33–40. doi: 10.1016/j.neuroimage.2013.01.039

Brian, J. E. (1996). Carbon dioxide and the cerebral circulation. *Anesthesiology* 81, 1308–1386. doi: 10.1097/00000542-199805000-00029

Bulte, D. P., Chiarelli, P. A., Wise, R. G., and Jezzard, P. (2007). Cerebral perfusion response to hyperoxia. *J. Cereb. Blood Flow Metab.* 27, 69–75. doi: 10.1038/sj.jcbfm.9600319

**AUTHOR CONTRIBUTIONS**

AB was responsible for all the data collection and data preprocessing. AC ran the data analysis and wrote the manuscript. AB and AC edited and reviewed the manuscript and prior to submission. Both authors contributed to the article and approved the submitted version.

**FUNDING**

This research was funded by an NWO talent scheme grant (VENI) awarded to AB.

**ACKNOWLEDGMENTS**

The authors would like to acknowledge collaborative support from H. Hoogduin, M. E. P. Philippens and J. C. W. Siero from the University Medical Center Utrecht in the Netherlands, as well as N. S. Coverdale and D. J. Cook from Queen's University in Kingston, Canada.

**SUPPLEMENTARY MATERIAL**

The Supplementary Material for this article can be found online at: https://www.frontiersin.org/articles/10.3389/fphys.2021.601369/full#supplementary-material

Champagne, A. A., Bhogal, A. A., Coverdale, N. S., Mark, C. I., and Cook, D. J. (2019). A novel perspective to calibrate temporal delays in cerebrovascular reactivity using hypercapnic and hyperoxic respiratory challenges. *Neuroimage* 187, 154–165. doi: 10.1016/j.neuroimage.2017.11.044

Champagne, A. A., Coverdale, N. S., Fernandez-Ruiz, J., Mark, C. I., and Cook, D. J. (2020). Compromised resting cerebral metabolism after sport-related concussion: a calibrated MRI study. *Brain Imaging Behav.* doi: 10.1007/s11682-019-00240-2 [Epub ahead of print].

Chester, M., Brien, J. O., Zappala, A., Cicirata, F., and Barrio, L. C. (2008). Regulation and Modulation of pH in the Brain. *Brain* 83, 1183–1221. doi: 10.1152/physrev.00010.2003

Claassen, J. A. H. R., Zhang, R., Fu, Q., Witkowski, S., and Levine, B. D. (2006). Transcranial Doppler estimation of cerebral blood flow and cerebrovascular conductance during modified rebreathing. *J. Appl. Physiol.* 102, 870–877. doi: 10.1152/japplphysiol.00906.2006

Conklin, J., Fierstra, J., Crawley, A. P., Han, J. S., Poublianc, J., Mandell, D. M., et al. (2010). Impaired cerebrovascular reactivity with steal phenomenon is associated with increased diffusion in white matter of patients with moyamoya disease. *Stroke* 41, 1610–1617. doi: 10.1161/STROKEAHA.110.579540

De Vis, J. B., Hendrikse, J., Bhogal, A., Adams, A., Kappelle, L. J., and Petersen, E. T. (2015). Age-related changes in brain hemodynamics; a calibrated MRI study. *Hum. Brain Mapp.* 36, 3973–3987. doi: 10.1002/hbm.22891

Donahue, M. J., Faraco, C. C., Strother, M. K., Chappell, M. A., Rane, S., and Hendrikse, J. A. H. R., Zhang, R., Fu, Q., Witkowski, S., and Levine, B. D. (2006). An analysis of the use of hyperoxia for measuring venous cerebral blood volume: comparison of the existing method with a new analysis approach. *Neuroimage* 72, 33–40. doi: 10.1016/j.neuroimage.2013.01.039

Brian, J. E. (1996). Carbon dioxide and the cerebral circulation. *Anesthesiology* 81, 1308–1386. doi: 10.1097/00000542-199805000-00029

Bulte, D. P., Chiarelli, P. A., Wise, R. G., and Jezzard, P. (2007). Cerebral perfusion response to hyperoxia. *J. Cereb. Blood Flow Metab.* 27, 69–75. doi: 10.1038/sj.jcbfm.9600319

Conklin, J., Fierstra, J., Crawley, A. P., Han, J. S., Poublianc, J., Mandell, D. M., et al. (2010). Impaired cerebrovascular reactivity with steal phenomenon is associated with increased diffusion in white matter of patients with moyamoya disease. *Stroke* 41, 1610–1617. doi: 10.1161/STROKEAHA.110.579540

De Vis, J. B., Hendrikse, J., Bhogal, A., Adams, A., Kappelle, L. J., and Petersen, E. T. (2015). Age-related changes in brain hemodynamics; a calibrated MRI study. *Hum. Brain Mapp.* 36, 3973–3987. doi: 10.1002/hbm.22891

Donahue, M. J., Faraco, C. C., Strother, M. K., Chappell, M. A., Rane, S., and Dethrage, L. M. (2014). Bolus arrival time and cerebral blood flow responses to hypercapnia. *J. Cereb. Blood Flow Metab.* 34, 1243–1252. doi: 10.1038/jcbfm.2014.81

Donahue, M. J., Strother, M. K., Lindsey, K. P., Hocke, L. M., Tong, Y., and de B Frederick, B. (2016). Time delay processing of hypercapnic fMRI allows quantitative parameterization of cerebrovascular reactivity and blood
metabolism. *J. Cereb. Blood Flow Metab.* 32, 1909–1918. doi: 10.1038/jcbfm.2012.93

Yoon, S. H., Zuccarello, M., and Rapoport, R. M. (2000). Reversal of hypercapnia induces endothelin-dependent constriction of basilar artery in rabbits with acute metabolic alkalosis. *Gen. Pharmacol. Vasc. Syst.* 35, 333–340. doi: 10.1016/S0306-3623(02)00112-X

Zhang, Y., Brady, M., and Smith, S. (2001). Segmentation of brain MR images through a hidden Markov random field model and the expectation-maximization algorithm. *IEEE Trans Med Imaging* 20, 45–57. doi: 10.1109/42.906424

**Conflict of Interest:** The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

*Copyright © 2021 Champagne and Bhogal. This is an open-access article distributed under the terms of the Creative Commons Attribution License (CC BY). The use, distribution or reproduction in other forums is permitted, provided the original author(s) and the copyright owner(s) are credited and that the original publication in this journal is cited, in accordance with accepted academic practice. No use, distribution or reproduction is permitted which does not comply with these terms.*