Vascular Modulation of Resting-State fMRI Functional Connectivity

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Abstract

Widely used in resting-state fMRI functional connectivity measurement (fcMRI), the BOLD signal is an indirect measure of neuronal activity, inherently modulated by vascular physiology. Notably, cerebrovascular reactivity (CVR) varies across individuals irrespective of neuronal changes, with unknown implications, compromising fcMRI interpretation. This work quantifies the relationship between CVR and resting motor fcMRI in healthy young adults. We modulate CVR within each individual, altering vascular tension through end-tidal CO\(_2\) (PETCO\(_2\)), and measure fcMRI during hypercapnic, hypocapnic and normocapnic states. PETCO\(_2\) significantly influenced CVR and fcMRI, but with different directionalities. Resting motor fcMRI was significantly positively associated with CVR across the group, however, the CVR-fcMRI relationship was highly subject dependent. Furthermore, the relationship was not mediated by BOLD signal fluctuation amplitude. This work demonstrates and quantifies a major vascular modulator of resting fcMRI among healthy individuals. We suggest that correcting for CVR in fcMRI is important in studying healthy and diseased brains.

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Contributions of Author

I am the co-first author of the manuscript on which chapters 2 through 5 are based. I have performed all of the work described, which includes:

- Contributions to experimental design and design refinement;
- Collection of experimental data; and
- Preprocessing and analysis of data.

All work was carried out under the supervision of Drs. J. Jean Chen and Stephen Strother. I have also contributed to the following peer-reviewed journal publications and conference abstracts.


Table of Contents

Contributions of Author................................................................................................................. iii
Table of Contents........................................................................................................................... iv
List of Figures ................................................................................................................................ vi
List of Abbreviations and symbols ............................................................................................... vii

1 Background and motivation........................................................................................................1
   1.1 Cerebrovascular reactivity .................................................................................................1
      1.1.1 Cerebrovascular reactivity and physiology .............................................................1
      1.1.2 Measuring cerebrovascular reactivity .......................................................................1
      1.1.3 Cerebrovascular modulation and carbon dioxide ..................................................2
   1.2 Resting-state functional connectivity based on MRI .....................................................5
      1.2.1 Measuring functional connectivity ..........................................................................5
      1.2.2 The BOLD fMRI technique ....................................................................................6
      1.2.3 Physiological origins of the BOLD signal ...............................................................8
      1.2.4 Sources of noise in resting-state fMRI .................................................................11
      1.2.5 Functional connectivity assessment techniques ................................................12
   1.3 CVR and functional connectivity measures .........................................................................14
      1.3.1 Vascular modulation of BOLD fMRI functional connectivity .............................14
      1.3.2 The role of noise in the vascular modulation of functional connectivity ..........15
      1.3.3 Hypothesis ................................................................................................................19

2 Methods..................................................................................................................................20
   2.1 Subject selection and ethical approval ............................................................................20
   2.2 Scanning ............................................................................................................................20
   2.3 Vascular manipulation .....................................................................................................21
      2.3.1 Vascular manipulation for CVR measurement ....................................................21
# List of Figures

Figure 1.1: Proton spins without and within a magnetic field ........................................................ 7

Figure 1.2: The BOLD hemodynamic response ............................................................................. 8

Figure 1.3: BOLD and physiological intermediates resultant of neuronal activity ...................... 9

Figure 1.4: Seed-based and ICA connectivity maps ..................................................................... 13

Figure 1.5: BOLD signal generation from neural and noise sources ......................................... 16

Figure 1.6: SNR dependence of measured connectivity ............................................................... 17

Figure 2.1: Experimental design ................................................................................................... 22

Figure 2.2: Sample delineation procedure for region of interest (ROI) for a typical subject ....... 23

Figure 3.1: Basal PETCO\textsubscript{2} modulations and corresponding PETO\textsubscript{2} levels ......................... 27

Figure 3.2: Group-average CVR, functional connectivity and amplitude of low frequency fluctuation (ALFF) maps .............................................................................................................. 28

Figure 3.3: Group-average CVR, functional connectivity and ALFF values across different capnic conditions ........................................................................................................................................ 28

Figure 3.4: The relationship between CVR and functional connectivity measurements ............ 29

Figure 3.5: The relationship between connectivity and ALFF ..................................................... 30

Figure 3.6: The relationship between ALFF and CVR ................................................................. 31
## List of Abbreviations and symbols

<table>
<thead>
<tr>
<th>Abbreviation</th>
<th>Description</th>
</tr>
</thead>
<tbody>
<tr>
<td>ACZ</td>
<td>Acetazolamide</td>
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<tr>
<td>ALFF</td>
<td>Amplitude of low-frequency fluctuations</td>
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<td>ANOVA</td>
<td>Analysis of variance</td>
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<td>ASL</td>
<td>Arterial spin labeling</td>
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<td>ATP</td>
<td>Adenosine triphosphate</td>
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<tr>
<td>B₀</td>
<td>Static magnetic field</td>
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<tr>
<td>B₁</td>
<td>Excitation magnetic field</td>
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<tr>
<td>BOLD</td>
<td>Blood oxygen level-dependent</td>
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<tr>
<td>CBF</td>
<td>Cerebral blood flow</td>
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<td>CBV</td>
<td>Cerebral blood volume</td>
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<tr>
<td>CMRO₂</td>
<td>Cerebral metabolic rate of oxygen</td>
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<tr>
<td>CO₂</td>
<td>Carbon dioxide</td>
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<tr>
<td>CSF</td>
<td>Cerebrospinal fluid</td>
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<tr>
<td>CT</td>
<td>Computed tomography</td>
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<td>CVR</td>
<td>Cerebrovascular reactivity</td>
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<td>DEF</td>
<td>Dynamic end-tidal forcing</td>
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<tr>
<td>dHb</td>
<td>Deoxyhemoglobin</td>
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<td>FA</td>
<td>Flip angle</td>
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<tr>
<td>fcMRI</td>
<td>Functional connectivity magnetic resonance imaging</td>
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<tr>
<td>fMRI</td>
<td>Functional magnetic resonance imaging</td>
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<tr>
<td>FOV</td>
<td>Field of view</td>
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<tr>
<td>FWHM</td>
<td>Full width at half maximum</td>
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<tr>
<td>GSR</td>
<td>Global signal regression</td>
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<tr>
<td>HRF</td>
<td>Hemodynamic response function</td>
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<tr>
<td>ICA</td>
<td>Independent component analysis</td>
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<tr>
<td>MNI152</td>
<td>Montreal Neurological Institute standard brain atlas</td>
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<tr>
<td>NO</td>
<td>Nitrous oxide</td>
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<td>NVC</td>
<td>Neurovascular coupling</td>
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<td>O₂</td>
<td>Oxygen</td>
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<tr>
<td>pCASL</td>
<td>Pseudo-continuous arterial spin labeling</td>
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<tr>
<td>PET</td>
<td>Positron emission tomography</td>
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<td>PETCO₂</td>
<td>Pressure of end-tidal carbon dioxide</td>
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<tr>
<td>PETO₂</td>
<td>Pressure of end-tidal oxygen</td>
</tr>
<tr>
<td>REB</td>
<td>Research ethics board</td>
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<tr>
<td>ROI</td>
<td>Region of interest</td>
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<tr>
<td>SNR</td>
<td>Signal-to-noise ratio</td>
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<tr>
<td>SPECT</td>
<td>Single positron emission computed tomography</td>
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<tr>
<td>T₁</td>
<td>Longitudinal relaxation time</td>
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<td>T₂</td>
<td>Transverse relaxation time</td>
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<tr>
<td>TCD</td>
<td>Transcranial Doppler ultrasonography</td>
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<tr>
<td>TE</td>
<td>Echo time</td>
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<td>TI</td>
<td>Inversion time</td>
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1 Background and motivation

1.1 Cerebrovascular reactivity

1.1.1 Cerebrovascular reactivity and physiology

Cerebrovascular reactivity (CVR) quantifies the change in blood flow in the brain in response to a vascular stimulus and is an important indicator of cerebrovascular health. CVR is associated with vascular reserve (Ito et al., 2003) and autoregulatory efficiency (Nur et al., 2009), each of which is a quantity related to the ability of blood vessels to act to maintain blood flow. Maintaining blood flow ensures an adequate supply of blood to the brain, which is critically important in preventing neuronal and glial cell injury and death (Attwell et al., 2010). While neurovascular coupling and functional hyperaemia describe the vascular response associated with neuronal activity, CVR provides an assessment of exclusively vascular ability independent of neuronal stimulus. CVR has been used to characterize disease states (Packard et al., 2003; Ziyeh et al., 2005), with reduced CVR demonstrated in cerebral steno-occlusive vascular disease (Mandell et al., 2011, 2008), lacunar infarction (Birns et al., 2009; Mandell et al., 2011), microbleeding (Birns et al., 2009; Conijn et al., 2012), and cognitive decline in aging and dementia (Hurford et al., 2014; Kastrup et al., 1998, p. 1998; Kovács et al., 2010; Suri et al., 2014).

CVR is known to vary within the population with the younger adults demonstrating greater CVR than older adults, and even within the older population CVR is shown to be correlated with maximal aerobic capacity, a gauge of aerobic fitness (Barnes et al., 2013).

1.1.2 Measuring cerebrovascular reactivity

There are a variety of ways to assess CVR, the choice of which will impact its application. Methods such as Xenon-enhanced X-ray computed tomography (CT), single positron emission computed tomography (SPECT), and positron emission tomography (PET) have been used as common imaging techniques for measuring CVR, however they require exposure to ionizing radiation and contrast agent injection, and are limited in their availability (Kassner et al., 2010). Another common method is transcranial Doppler ultrasonography (TCD). TCD is an imaging modality for making real-time measurements of blood flow velocity in large arteries non-invasively, employing no ionizing radiation. TCD is inexpensive and portable, making it
particularly useful to individuals for whom more inflexible imaging modalities are excessively onerous or impossible. TCD has been established as a reproducible and reliable measure (Demolis et al., 1993). TCD is however limited in that it only provides blood flow velocity from a single artery at a time (typically the middle cerebral artery), providing a whole-brain measure of CVR without any assessment of the spatial distribution of vascular reactivity in the brain. Further, it is heavily reliant on the skill and ability of the operator as well as individual anatomical properties that permit ultrasonic penetration through the skull while orienting the ultrasonic transducer properly relative to blood vessels. Further, in measuring blood velocity and not volumetric flow, TCD lacks vital information for characterizing the dynamics in assessing CVR.

fMRI is increasingly used to assess CVR (Kassner et al., 2010), providing a non-invasive tomographic technique that measures spatially resolved CVR without the use of ionizing radiation. CVR can be assessed by fMRI using either BOLD contrast or an arterial spin labelling (ASL) technique. ASL techniques can be used to quantify CBF more directly than BOLD but suffers from lower SNR, and is particularly sensitive to effects of timing relating to transit time of the labelled blood which can pose an issue when the system is subject to vascular stimulus or in individuals with vascular impairments. Further, ASL MRI pulse sequences are not as readily available as those of BOLD, and thus BOLD is the more prevalent approach. BOLD fMRI CVR is as well a reproducible and reliable measure (Kassner et al., 2010). BOLD does not directly measure CBF but the relative fractional magnitudes of CBF changes to oxygen metabolism, where we expect only nominal changes in oxygen metabolism under vascular stimulus.

1.1.3 Cerebrovascular modulation and carbon dioxide

Measurement of CVR necessarily requires vascular stimulus. Existing stimuli for CVR mapping can be categorized into either mechanical or chemical vascular manipulations. Mechanical manipulation of arterial pressure is typically a reduction in arterial blood pressure accomplished using thigh cuff release (Aaslid et al., 1989) or lower body negative pressure (Tan, 2012). These techniques are often inappropriate in populations with vascular disease, and can induce variable effects even within a healthy population, which could be even further variable in effect in older or diseased populations.
Chemical vascular manipulations for CVR measurement are typically achieved with acetazolamide (ACZ) or CO$_2$. ACZ is a carbonic anhydrase inhibitor that produces acidosis that results in the relaxation of vascular smooth muscle. A typical ACZ dose is 1 g delivered intravenously, although higher doses may be used for purposes of eliciting a maximal vasodilatory response, which will require a mass-rated dose in excess of 15 mg/kg (Dahl et al., 1995). It only requires a single dose, no task, and is quite safe. However, in addition to the invasiveness of the injection, standardization of dosage across participants can be difficult due to individual variation in size and dose delivery with variations in the resulting time-to-peak, duration, and elimination rate. Further, ACZ administration can result in participant discomfort that is not easily or quickly mitigated.

CO$_2$ is a vasoactive compound that acts on blood vessels via the arterial baroreflex (Ainslie et al., 2008). The vascular response to CO$_2$ has been well established using transcranial Doppler ultrasound of arterial blood flow (Battisti-Charbonney et al., 2011). For instance, increased arterial CO$_2$ stimulates release of endothelium-derived relaxing factor nitric oxide (NO). NO is a compound with important and widespread action in regulating cerebral circulation and is a mediator of the cerebrovascular dilation in response to CO$_2$ (Iadecola, 1992). While it is not the sole factor involved, its action is particularly important at moderate levels of CO$_2$ (Iadecola and Zhang, 1994), which is used in the majority of CO$_2$-related vascular experiments. Particularly relevant to fMRI, the BOLD-measured functional hyperaemia response to neuronal activation is understood to be due in a significant part to NO, which is released in response to astrocyte-mediated glutamate signaling (Attwell et al., 2010).

CO$_2$ is a potent, endogenously occurring vasodilator easily administered through ventilation. Changes in arterial CO$_2$ are easily monitored in the breath as they are linear with end-tidal CO$_2$ (Battisti-Charbonney et al., 2011). CO$_2$ is generally well tolerated, repeatable, and safe to use even in diseased populations (Mandell et al., 2008). Formerly, CO$_2$ modulation was viewed disadvantageously as it required cooperation from a participant to either hold their breath or to hyperventilate, but this is no longer the case with the increasing prominence of alternative ventilation techniques and technologies (Fierstra et al., 2013). Techniques for CO$_2$ manipulation include breath holding, hyperventilation, CO$_2$ inspiration, rebreathing, dynamic end-tidal forcing (DEF), and prospective end-tidal targeting.
Prospective end-tidal targeting is the most comprehensive method, resolving many of the shortcomings inherent in alternative methods. Breath hold and hyperventilation paradigms require a participant to perform a ventilatory maneuver, resigning them to the active performance of a task, introducing confounding effects to the desired vascular stimulation. Further, all methods except for DEF and prospective end-tidal targeting are subject to significant variance when it comes to their actual CO$_2$ modulations, as a result of participant ability, including a wide range of anatomical and physiological variations, both vascular and ventilatory, making these methods unsuitable for reproducible CVR measurements (Fierstra et al., 2013).

DEF and prospective end-tidal targeting are computerized methods for delivering gasses to a participant for producing CO$_2$ changes for assessing CVR. DEF falls short of prospective end-tidal targeting for a number of reasons, specifically its use of a feedback mechanism for adjusting administration of gasses, which is resource intensive and slow to make the appropriate adjustments, and though safety measures are in place, in the event of system failure, DEF could expose a participant to completely anoxic ventilation, which is dangerous (Fierstra et al., 2013).

Prospective end-tidal targeting allows administration of reliable changes in CO$_2$ while maintaining a constant level of O$_2$, representing a valid alternative to non-computerized methods with the considerable advantage of minimizing O$_2$-related confounds innate to other methods (Tancredi and Hoge, 2013). The precise control over both arterial CO$_2$ and O$_2$, allows assessment of CVR to become more stable and predictable (Mark et al., 2010). The system is MRI-compatible, compact, economical, and safe. Using a 3-valve manifold, breathing circuit, and computerized control of administered gases, sequential delivery of inspired and previously exhaled gas is achieved. The method allows for independent control of CO$_2$ and O$_2$ based on feedforward prospective methods eliminating the need for feedback correction mechanisms and computations, and requires minimal cooperation on the part of the subjects. While the prospective end-tidal targeting system has many advantages, its dependability relies on having accurate physiological information about a subject such as baseline CO$_2$, and rates of CO$_2$ production and O$_2$ consumption.

It had been demonstrated that under the influence of graded hyper- and hypocapnia at mild levels, there are no expected global CMRO$_2$ changes (Chen and Pike, 2010a). However, there is evidence for a possible decrease in CMRO$_2$ in hypercapnia (Xu et al., 2011) at higher levels of
CO₂ manipulation, which calls for additional work to further understand the effect that CO₂ stimulus has on metabolic and neuronal activity.

1.2 Resting-state functional connectivity based on MRI

1.2.1 Measuring functional connectivity

Functional connectivity describes the temporal synchrony of activity measured in spatially distributed regions. This temporal synchrony implies an underlying collaboration of separate features towards a given function or task, and allows the identification of distinct collaborating neuronal populations termed “networks” (Damoiseaux et al., 2006). Functional connectivity in the resting state was first observed using MRI in the motor cortex, using blood-oxygen level dependent (BOLD) functional magnetic resonance imaging (fMRI) (Biswal et al., 1995). This phenomenon is consistent, robust, and reliable (Shehzad et al., 2009; Zuo et al., 2010). Resting-state functional connectivity has as well been associated with a myriad of brain functions including vision, memory, sensory and motor processing, (lateralized) executive control, salience, and default-mode brain activity. Moreover, functional connectivity changes have been shown in aging (Hedden et al., 2009), as well as various disease states including Alzheimer's disease and other dementia pathologies (Allen et al., 2007; Greicius et al., 2004; He et al., 2007; Kenny et al., 2012; Liu et al., 2008; Seeley et al., 2009). Resting-state measures are particularly valuable to clinical applications, as they do not require any additional apparatus or the performance of any overt task, which can be difficult for diseased and elderly populations. The motor networks is important in functional connectivity studies not only historically as the first observed network using resting-state fMRI, but also as it can be observed in a single imaged plane, is among the most robustly observed networks, and can otherwise be validated quickly with simple motor tasks.

Two decades after the initial discovery of resting-state fMRI functional connectivity, resting-state functional connectivity is still assessed predominantly using BOLD fMRI. BOLD fMRI is a noninvasive imaging modality that does not expose subjects to ionizing radiation, and provides spatially resolved images of the brain with sufficient temporal resolution to investigate the dynamics of the hemodynamic response to brain activity. In fMRI, images are taken consecutively, often evenly sampled in time, allowing investigation of temporal changes in brain
activity. Furthermore, BOLD contrast is intrinsic and thus requires no administration of exogenous contrast agents, and is described in more detail in the following sections.

1.2.2 The BOLD fMRI technique

The BOLD signal reflects the amount of deoxyhemoglobin (dHb) in the blood, which attenuates signal due to its paramagnetic nature. Functional MRI (fMRI), like other MRI modalities, relies on the measurement of magnetic spins in a magnetic field to produce images, specifically spins associated with the \(^1\)H hydrogen atom, which is abundant in water, which dominates the composition of biological tissues. \(^1\)H consists of a single unpaired proton, which has intrinsic magnetic spin (moment). When exposed to an external static magnetic field, the spin becomes quantized with spins aligning either parallel to the magnetic field or antiparallel. A greater number of spins will align with the external static field (\(B_0\)), while fewer will orient themselves opposite the field in a state of higher energy. The amount of anti-aligned spins increases with temperature, which serves to increase the total system energy. Moreover, the spins are not statically aligned but rather precess about the \(B_0\) direction, at a frequency defined by the particle, in this case a proton, and the magnetic field strength. This frequency is called the Larmor frequency. The Larmor frequency equivalently prescribes the frequency of a photon whose energy is equal to difference in energies between the aligned and anti-aligned states. Furthermore, the population of spins exposed to the field precesses in randomly distributed phase, and over a population of atoms the phases of their individual precession is negligible and a bulk magnetic moment parallel to the applied field will prevail.

The bulk magnetic moment generated by the \(B_0\)-alignment can then be perturbed by an external magnetic field applied perpendicular to the magnetic field, exerting a torque, rotating the direction of the bulk magnetic moment. This process is termed “excitation,” and the excitation field referred to as \(B_1\). Once the bulk magnetic moment is no longer parallel to the external field, however, it will begin to precess about the \(B_0\) field's direction, again at the Larmor frequency. If we wish to rotate the magnetic moment further away from the direction of \(B_0\), the applied \(B_1\) field should always be applied perpendicular to the magnetic moment, following the left-hand rule. In summary, the magnetic moment can be most effectively manipulated by a \(B_1\) field whose direction in the plane perpendicular to \(B_0\) is rotating at the Larmor frequency.
Figure 1.1: Proton spins without and within a magnetic field. A) Atomic spins and their magnetic dipole moments randomly oriented in free space. B) Atomic spins in a magnetic field indicated by \( B_0 \) vector, precess around this direction at the Larmor frequency with aligned spins (blue) slightly outnumbering anti-aligned spins (red).

Once the magnetic moment is disturbed from its equilibrium orientation, it will precess about \( B_0 \) while returning to this equilibrium, the lowest energy state configuration orientation. MRI is based on measuring how long the magnetic moment takes returning to its equilibrium state, which occurs at different rates in different tissues. The parameter \( T_1 \) describes the exponential recovery of magnetization in the direction of the \( B_0 \) field. Further, the exponential decay of magnetization from the plane normal to \( B_0 \) can be assessed. Magnetization leaves the transverse plane primarily through two mechanisms: spin-spin interaction, and precession frequency changes due to field inhomogeneity. The parameter \( T_2 \) properly describes the loss of magnetization due to spin-spin interactions while \( T_2^* \) describes losses due to spin dephasing caused by inhomogeneity in precession frequency.

\( T_2^* \) is the dominant contrast mechanism employed in BOLD fMRI. As mentioned, it provides contrast based on signal loss due to dephasing in tissues with particularly inhomogeneous magnetic fields. This effect is very pronounced as a result of present deoxyhemoglobin (dHb), which is what BOLD fMRI is primarily assessing. dHb is paramagnetic, meaning that it possesses positive magnetic susceptibility. That is, when exposed to an external magnetic field, dHb will align its own magnetic moment with the external field, creating a non-negligible locally significant field, creating greater field inhomogeneity. Thus increased local dHb perturbs the
local magnetic environment experienced by $^1$H spins, resulting in decreased local MRI signal. Then, after a duration termed the “echo time” (TE), the transverse magnetization is measured.

The choice of TE will be important to finding a compromise between signal magnitude and contrast. There is also the matter of choosing how frequently to collect images, which is determined by the prescribed repetition time (TR). TR will affect fMRI in two ways. First, the sampling rate will determine in a fundamental mathematical sense what dynamics we are able to measure without unrecoverable loss to aliasing. Second, subsequent excitations will be applied to a system that has not completely returned to equilibrium. Choosing TR will be important to generating sufficient signal once a steady-state between consecutive excitation and relaxation periods has been reached. In the same pursuit of choosing TR, we can also choose by how much to excite the system each time, described as flip angle (FA) which is the amount that magnetization is perturbed from equilibrium prescribed by the resultant angle formed between the magnetization and $B_0$.

1.2.3 Physiological origins of the BOLD signal

As mentioned earlier, BOLD fMRI relies on measuring dHb in the venous section of the systemic circulation. The arterial portion of cerebral vasculature is uniformly highly oxygenated, but oxygenation begins to decrease through the arterioles, capillaries, and venules, finally being collected into large veins. It is only in this partially deoxygenated circulation that we can begin to investigate the spatial distribution in dHb. dHb present in the venous circulation is the product of metabolism, specifically aerobic metabolism in which oxygen from oxyhemoglobin is used to convert glucose to adenosine triphosphate (ATP), the primary source of energy for the brain.

![Figure 1.2: The BOLD hemodynamic response](image)

A schematic of the BOLD response to neuronal stimulus demonstrating the fast negative response (~2 s post-stimulus), the main BOLD response (peak ~5 s post-stimulus), and post-stimulus undershoot with recovery to baseline (~1 min) (Norris, 2006).
The increased production of dHb as the result of aerobic metabolism is associated with increased neuronal activity, hence increased cerebral metabolic rate of oxygen (CMRO$_2$) produces elevated dHb that attenuates BOLD signal. However, increased neuronal activity is not associated with decreased BOLD signal, but quite the contrary. This is due to a number of physiological factors that affect the BOLD signal, of primary interest being cerebral blood flow (CBF) and cerebral blood volume (CBV), both of which increase with increased neuronal activity. Typically, the CBF increase associated with brain activity far exceeds the fractional CMRO$_2$ increases, serving

![Figure 1.3: BOLD and physiological intermediates resultant of neuronal activity.](image)

**Figure 1.3: BOLD and physiological intermediates resultant of neuronal activity.** A flowchart describing the ordered of events through which the BOLD signal results from changes in neuronal activity. Neurovascular coupling (NVC) is shown as the grey arrow, describing the blood flow (CBF) and blood volume (CBV) changes that occur as a result of neuronal activity. Arrows between steps do not imply causality, but simply order, for example, CBF changes are directly causal of neuronal activity and not O$_2$ changes, but they occur temporally following changes in tissue energy demand and in consumption of glucose and O$_2$. The dark blue versus light blue boxes describe how each of the parameters changes are correlated or anti-correlated. Increases in neuronal activity are associated with increases in other dark blue factors but decreases in light blue factors, whereas decreases in neuronal activity are associated with decreases in dark blue factors but increases in light blue ones.
the function of diluting the venous dHb. On the other hand, rising CBV, which coexists with increasing CBF, increases the amount of venous dHb occupancy simply by increasing the volume of total present blood. These processes follow distinct dynamics, resulting in the BOLD hemodynamic response.

According to the published BOLD response function, the BOLD signal decreases slightly in the first 1-2 seconds as increases in CMRO$_2$ dominate, followed by much larger increase in BOLD signal peaking at around 5 seconds, about 4 seconds FWHM, as CBF effects dominate both CMRO$_2$ and CBV. Following this, the signal returns to baseline as these parameters do with a slight post-stimulus undershoot, which is task dependent (Chen and Pike, 2009a). The increase in CBF which causes increased BOLD signal is the result of feed-forward signaling associated with neuronal activity termed functional hyperaemia or neurovascular coupling (NVC). This process is essential for the existence of fMRI, but also introduces vascular ambiguity into the BOLD signal.

The complex relationship between these physiological parameters that gives rise to the BOLD signal can be summed up as follows in the dHb dilution model (Davis et al., 1998; Hoge et al., 1999).

$$\frac{\Delta BOLD}{BOLD_0} = M \left(1 - \left(\frac{CMRO_2}{CMRO_{2,0}}\right)^\beta \left(\frac{CBF}{CBF_0}\right)^{\alpha-\beta}\right)$$

[1.1]

where the fractional BOLD signal change is described with subscript “0” denoting baseline. $M$ is a scaling factor that can be written as $M = A \cdot CBV_0 \cdot TE \cdot [dHb]_0^\beta$, where $A$ is a scaling factor that depends on $B_0$ and $[dHb]$ denotes the blood concentration of dHb. $\alpha$ and $\beta$ are constants, experimentally determined, but are well approximated by $\alpha = 0.2$ (Chen and Pike, 2009b, 2010a), $\beta = 1.5$ (Buxton et al., 2004; Uludag et al., 2004). It is important in understanding the signal model description of BOLD changes, that with the exception of CMRO$_2$, all other dynamics contributing to the BOLD signal are fundamentally vascular. In this formalism, it becomes clear that the generation of BOLD signal is dependent on the relative magnitudes in the fractional changes of CBF and CMRO$_2$. CMRO$_2$ provides the most direct correlate of activity for a group of neurons (Hyder and Blumenfeld, 2004). At low amplitudes, the relationship between BOLD and CBF responses is approximately linear (Hoge et al., 1999).
1.2.4 Sources of noise in resting-state fMRI

An understanding of the underlying sources and structures of noise have allowed for the development of methods and techniques for reducing its obscuring of BOLD signal of interest. For a typical gradient-echo EPI rs-fMRI imaging protocol at a magnetic field strength of 3 T, physiological noise will outweigh other sources of noise (Kruger and Glover, 2001). However, thermal noise and motion constitute major non-physiological noise sources whose effects are non-negligible, particularly for rs-fMRI.

Physiological noise is any noise resulting from innate physiological processes, primarily cardiac pulsation and respiration. These processes are also likely to result in bulk motion that is typically periodic and predictable. Cardiac pulsation primarily affects areas with high blood or CSF volume (Birn et al., 2006) and induces fluctuations in the BOLD signal correlated to itself, due to both blood composition and tissue motion. Respiration has a multitude of effects on the BOLD signal. Firstly, respiration causes bulk motion in the head and brain. Then, there is the effect of the changing lung volume during respiration, which alters the bulk magnetic susceptibility in the scanner dynamically (Van de Moortele et al., 2002), seemingly shifting the brain, or producing more subtle changes still. Respiration also produces its own physiological changes related to the depth and rate of breathing that will produce changes in the BOLD signal. These physiological changes are most evident in grey matter and large vessels and are related to the resulting changes in vascular CO$_2$ (Birn et al., 2006).

Further, there is signal noise related to motion. Over the course of an fMRI experiment, the head position of a participant within the head coil is likely to change, albeit minutely, resulting in bulk motion of the brain image in fMRI data. The effect of motion is mostly perceived in movement between consecutive functional volumes, but there is also motion occurring between excitation and echo readout of a single volume, which will affect measured BOLD signal.

Additionally, thermal noise affects any MRI modality, adding random noise in proportion to temperature as a result of random Brownian motion of electrons, both in the imaged subject and in the scanner and system electronics.

Images must be taken sufficiently quickly to allow appropriate representation of these physiologic processes without aliasing, which is the misrepresentation of high-frequency signals.
as low-frequency signals as a result of too low a sampling frequency. If this is achieved, the noise can be minimized, improving the specificity of the data to reflect neuronal activity.

1.2.5 Functional connectivity assessment techniques

There are two main categories of techniques for assessing resting-state fMRI functional connectivity, seed-based analyses and data-driven methods. Seed-based methods were the first used (Biswal et al., 1995). A seed region is chosen typically based on prior knowledge and anatomical landmarks, and a mean time-course of voxels within that seed region is computed. Connectivity maps are commonly computed by assigning each voxel the Pearson product-moment correlation coefficient (commonly “correlation” or “correlation coefficient”) between its own time course and the seed time-course.

Data-driven methods do not necessarily require prior knowledge of seed locations. The most common method is independent component analysis (ICA). ICA decomposes all voxel time-courses into a set of statistically independent time-courses and spatial maps (Kiviniemi et al., 2003). These independent time-courses are then assigned to different functional networks. More knowledge will be necessary still to interpret and identify and interpret networks. ICA may also comprise noise removal, as physiological signals such as respiration and cardiac pulsation are spatially extensive and temporally distinct. It has been demonstrated that seed-based analyses can be equivalently expressed as linear combinations of within- and between-network connectivity of ICA-derived networks (Joel et al., 2011).

There are also a variety of techniques that can be used to correct for these physiologic noises in the context of resting-state fMRI. Regression of physiological signals is such a tool to remove these nuisance signals. Regressors are generated either from mean time-courses in cerebrospinal fluid (CSF) and white matter, or from external physiological measurements of cardiac pulsation and respiration (Birn et al., 2006; Chang and Glover, 2009; Fox et al., 2009). There is concern however, that physiologic regressors cannot be outright discounted and removed, especially in cases where experimental effects modulate respiration and/or cardiac processes.

Another more broad proposed method is global signal regression (GSR), which is the removal of the mean time-course of all voxels (including grey matter, white matter and cerebrospinal fluid). GSR involves generation of a mean time course from all voxels, and regression from every
Figure 1.4: Seed-based and ICA connectivity maps. Comparing connectivity maps from 4 different networks generated using seed-based (left column) and independent component analysis (ICA, right column) methods on the same data set. Seed-based maps are from motor, visual, posterior cingulate, and intraparietal cortices respectively. From each technique the results over the networks shown are convergent (van Dijk et al., 2010).
voxel’s time course by way of subtracting the voxel’s projection on to the mean time course, or equivalently, orthogonalization to this mean time course. While it has been demonstrated to improve the predictive ability of functional connectivity measures (Fox et al., 2009), it induces negative correlations that otherwise would not be found and are thus of unclear validity. This is argued in the case of negative correlations between the default-mode and task-positive network, which is reported both with and without physiological noise correction, although greater in its absence (Chang and Glover, 2009). Concurrently it is also shown that physiological noise correction and regression of white matter and CSF signals can increase negative correlations and decrease positive ones, particularly in the default-mode network, possibly explained by the similarities that have been observed between physiological noise regressors and global signal (Chang and Glover, 2009).

It has been noted that removal of changes in respiration improves the identification of task-related signal changes (Birn et al., 2006).

1.3 CVR and functional connectivity measures

1.3.1 Vascular modulation of BOLD fMRI functional connectivity

The BOLD fMRI signal is used as a proxy for neuronal activity, permitting assessment of BOLD fMRI functional connectivity as a surrogate measure of synchronous neuronal connectivity. As a result, changes in neuronal connectivity will result in changes to BOLD functional connectivity. However, since BOLD fMRI measurements do not reflect neuronal activity directly, multimodal techniques involving EEG and/or MEG are required to further investigate neuronal activity more directly to understand its role in BOLD connectivity.

While BOLD signal reflects the hemodynamic result of neural activity (Buxton et al., 2004), reduced neurovascular coupling can also decrease BOLD connectivity even in the absence of decreased neuronal connectivity (Liu, 2013). In Alzheimer's disease, decreased connectivity in resting-state networks is observed (Allen et al., 2007; Brun and Englund, 1986; Greicius et al., 2004; He et al., 2007; Li et al., 2002; Liu et al., 2008; Sorg et al., 2007; Wang et al., 2007, 2006), however, we are to expect that in the disease state, there are concurrent decreases in both neuronal connectivity and neurovascular coupling (Iadecola, 2004). Drugs, anaesthesia, medication, and individual physiological variation can each cause changes in neurovascular
coupling to modulate measured connectivity (Greicius et al., 2008; Khalili-Mahani et al., 2012; Kiviniemi et al., 2005; Li et al., 2000; Peltier and Shah, 2011; Wong et al., 2012). Further, changes in the BOLD response can be accounted for by biomechanical changes in the vasculature without changes in neuronal activity (Liu, 2013). CVR is culpable in this effect as reduced vascular responsiveness is associated with reduced amplitude and slower dynamics of BOLD signal (Behzadi and Liu, 2005; Liu et al., 2004; Rack-Gomer and Liu, 2012).

Neurovascular coupling (NVC) describes how the BOLD signal represents the underlying neuronal activity, prescribing factors such as the shape of the BOLD response and its spectral frequency distribution, the magnitude of the response and the time delay associated with the BOLD response. Changes in NVC can alone modulate BOLD functional connectivity and further, changes in connectivity for such a NVC modulation will be most pronounced when these changes apply selectively to only some regions and not others. Even if neural fluctuations in two regions are highly correlated, applying different hemodynamic responses to the two can significantly modulate connectivity, even in the absence of noise considerations. This can occur in response to pharmacological factors such as alcohol where the hemodynamic response modulations are region-dependent (Luchtmann et al., 2010).

1.3.2 The role of noise in the vascular modulation of functional connectivity

The BOLD signal suffers from noise and nuisance contributions, which will interfere with assessment of functional connectivity, as depicted in Figure 1.5. The neural activity in two distinct regions are correlated with correlation constant of $\rho$, following which, neurovascular coupling (NVC) produces BOLD signal causally related to the neuronal activity. The BOLD signals in these regions produced as a direct result of neural activity and NVC has theoretical correlation $r_s$, in the absence of noise effects. The actual measured correlation $r$ is computed after signals have acquired noise contributions of non-neuronal signals. In reality, there is no precise distinction between these many factors that produce the measured BOLD signal, and unraveling the role and contribution of each is critically important to understanding BOLD functional connectivity.

In Figure 1.5 in region 2, there are two different HRF functions shown for the role of NVC where each has the same shape but they are scalar multiples of one another. The signals ($s_2$) that they then generate are scalar multiples as well. While mathematically, the correlation between
two signals is identical under scaling by a constant, connectivity is sensitive to this effect due to the presence of noise in the measured signals \((x_1 \text { and } x_2)\). Modulation of connectivity due to signal scaling is fundamentally important to understanding the effects that noise has on connectivity. The effect is summarized in the signal-to-noise ratio (SNR), which is the ratio of signal \((s_1 \text { or } s_2)\) variance to noise \((n_1 \text { or } n_2)\) variance. SNR will determine to what extent the measured connectivity reflects correlation of meaningful signal versus correlations of the noise.

**Figure 1.5:** BOLD signal generation from neural and noise sources. Connectivity between two regions depends on neurovascular coupling of neural fluctuations and noise in each region. In the resting state, neural fluctuations between two regions have correlation \(\rho\) and produce BOLD signals \(x_1\) and \(x_2\) with correlation \(r\). The signals \(x_1\) and \(x_2\) can be interpreted as the sum of signal produced as the result of neural power fluctuations \((s_1 \text { and } s_2)\) and other signals, termed noise \((n_1 \text { and } n_2)\). The signals \(s_1\) and \(s_2\) have correlation \(r_s\), although the measured correlation \(r\) will suffer noise contributions. The effect of neurovascular coupling is represented by hemodynamic response functions (HRF) whose convolution with neural power fluctuations produce the signals \(s_1\) and \(s_2\). The changes in NVC in region 1 are shown by the slowing of hemodynamic response, both a delay and a smoothing, of the black compared to the blue HRF producing the black and the blue signals \(s_1\), respectively. NVC changes in region 2 are shown by a decrease in magnitude of the green HRF compared to the red, producing respective \(s_2\) signals. Adapted from (Liu, 2013).
The measured correlation $r$ as a function of SNR is given as

$$r = r_s \frac{SNR + (r_n/r_s)}{SNR + 1}$$  \[1.2\]

where $r_n$ is the correlation of the noise and $r_s$ is the correlation of the neuronal BOLD signal (Liu, 2013). Hypothetically, if SNR is identically zero, the measured correlation is strictly the correlation of the noise, while as SNR tends to infinity the measured correlation will tend towards the correlation of the neuronal signal. In Figure 1.5 in region 2, where the green HRF has the same shape as the red HRF but is scaled by a factor of 1/3, the resultant $s_2$ signal then is as well scaled by 1/3. Thus the SNR is scaled by 1/9, shifting the measured connectivity towards $r_n$ away from $r_s$. This is illustrated in Figure 1.6 showing curves of measured correlation $r$ as a function of SNR. The solid blue shows the case of $r_n = 0.7$ and the dashed green shows $r_n = 0.3$, with $r_s = 0.5$ for both curves.

**Figure 1.6: SNR dependence of measured connectivity.** Measured correlation $r$ plotted as a function of SNR as per Equation [1.2]. The relationship is dependent on the correlation of $r_s$, the BOLD signal produced from hemodynamic response to neuronal activity; and $r_n$, the correlation of the noise signal. Two cases are shown, with noise correlation less than ($r_n = 0.3$, dotted green) and greater than ($r_n = 0.7$, solid blue) the correlation of neuronal signal ($r_s = 0.5$) (Liu, 2013).
Possible changes that affect the measured connectivity can be understood within the framework of Equation [1.1]. Changes in baseline CBF can alter amplitude and shape of BOLD response (Cohen et al., 2002), and have been of great interest as they are related to wide range of physiological, pharmacological, and disease-related vascular states. Baseline CBF changes will affect the dynamics of the signal to CBF changes as shown in the CBF/CBF$_0$ term. If CBF is increased, further changes in CBF have less of an effect on the BOLD signal and if CBF is decreased, further CBF changes will have a more significant effect changing the BOLD signal. Also, CBF will induce scaling changes in the BOLD signal response. Primarily, it has been suggested that increased CBF can decrease the BOLD signal by decreasing [dHb] due to washout, noting that [dHb] is a linear scaling factor in Equation [1.1] (Liu, 2013). There is also the possibility that some of this [dHb] scaling is diluted by a concurrent increase in CBV due to increased CBF, as CBV is also part of signal scaling in Equation [1.1]. CBF changes affecting BOLD response might manifest as a scaling to HRF occurring in NVC as shown demonstrated in region 2 in Figure 1.5. While Equation [1.1] was originally developed to explain task-related BOLD fMRI, evidence suggests that this BOLD signal model provides the framework for understanding, explaining, and interpreting the resting-state BOLD signal as well (Liu, 2013). Similarly, it applies to BOLD fMRI measurements of CVR. In CVR measurements it quantifies the BOLD signal response to CBF changes, which are the same as the effect that drives neural generation of BOLD signal. CVR then ought to as well describe BOLD signal amplitude though the same equation, meaning that CVR will play a role in the resultant SNR and thus BOLD fMRI measurement of functional connectivity.

Given that CVR directly influences the amplitude of task-related and resting-state fMRI signals alike, a potential surrogate for quantifying vascular effects in rs-fMRI is the amplitude of low-frequency fluctuations (ALFF), computed voxel-wise as the area under the power spectrum of the rs-fMRI signal. The ALFF contains contributions from neural activity, vascular modulators and noise. The preprocessing, most importantly, restricts the signal to its low-frequency components, which are of particular interest as they are suspected to be most specifically reflective of signal that is generated by the hemodynamic response to neuronal activity. The ALFF has been shown to positively correlate with BOLD signal (Liu et al., 2012), and ALFF variability has been shown to be a correlate of network connectivity strength, potentially due to contributions from neuronal activity (Di et al., 2013). ALFF has also been shown to reduce
within- and between-subject variability when used as a scaling factor in task studies (Biswal et al., 2007; Kannurpatti et al., 2011) and thus proves to be a promising surrogate to CVR (Kannurpatti et al., 2014), bridging vascular BOLD effects and functional connectivity values.

While it is clear that the value of $r_s$, the neuronally specific connectivity, will affect the measured connectivity $r$, the signals $s_1$ and $s_2$ are directly causal of neuronal activity and NVC, so modulations in $r_s$ will be caused by some underlying changes in one of these factors. Changes in noise connectivity $r_n$ can also affect measured connectivity. This can be demonstrated in Figure 1.6 comparing the two curves with the same value of $r_s$ and different values of $r_n$. The lower than SNR, the more pronounced the difference between each pair. In reality, the magnitude of the noise contribution is never explicit or clear and the true SNR of the measured rs-fMRI BOLD signal is not knowable. Understanding the role of non-neural contributions in the presence of noise is important to interpreting our results.

1.3.3 Hypothesis

We hypothesize that resting-state fMRI functional connectivity is positively associated with fMRI-assessed cerebrovascular reactivity. As described earlier, CVR is quantified through the reactivity of the BOLD signal specifically to CO$_2$, but the mechanisms by which CO$_2$ acts on the vasculature involve the same mechanisms by which BOLD signal is generated by neuronal activity. Then CVR is suspected to be an appropriate measure to assess BOLD sensitivity to neuronal activity, of and thus functional connectivity driven by neuronal activity may have the same dependence.
2 Methods

The following chapters 2 through 5 are based on a manuscript submitted for peer review, which is under revision as of submission of this thesis.* We focused on a group of healthy young adults, as our aim in this work is to quantify a biomechanically driven relationship between CVR and rs-fMRI functional connectivity without confounding neurological or cerebrovascular diseases.

2.1 Subject selection and ethical approval

We studied 18 healthy participants, (10 men, 8 women), aged from 18 to 32 years (mean = 26.7 years, SD = 4.3). Participants were recruited through the Baycrest Participants Database, consisting of individuals from the Baycrest and local communities. The study was approved by the research ethics board (REB) of Baycrest, and the experiments were performed with the understanding and written consent of each participant, according to REB guidelines.

REB approval is found in Appendix A1.

2.2 Scanning

All images were acquired using a Siemens TIM Trio 3 Tesla System (Siemens, Erlangen, Germany). The scans employed 32-channel phased-array head coil reception and body-coil transmission. Resting-state BOLD fMRI data was acquired using a gradient-echo EPI pulse sequence (TR = 380 ms, TE = 30 ms, FA = 40°, 7 slices, 3.44×3.44×6.25 mm³, 950 volumes). BOLD-based CVR was measured using the second echo of a dual-echo pseudo-continuous ASL (pCASL) sequence (TR = 3500 ms, TEBOLD = 25 ms, FA = 90°, 20 slices, 3.44×3.44x6 m³, 120 volumes). The pCASL data was also used in a separate publication concerning CVR measurement (Halani et al., 2015). Moreover, a 2-minute motor-functional localizer was collected using gradient-echo EPI BOLD, during which the subject was instructed to repeat blocks of bilateral finger flexion. The finger-flexion related region was the target region for the subsequent analyses to avoid the potential bias of respiratory tasks on motor connectivity in

breathing-related motor regions. Moreover, a $T_1$-weighted MPRAGE anatomical image was acquired (TR = 2400 ms, TE = 2.43 ms, FOV = 256 mm, TI = 1000 ms, readout bandwidth = 180 Hz/px, voxel size = 1x1x1 mm$^3$).

2.3 Vascular manipulation

All vascular manipulations were achieved by administering mixtures of $O_2$, $CO_2$ and medical air delivered using the RespirAct$^{TM}$ breathing circuit (Thornhill Research, Toronto, Canada), designed to provide computerized and independent targeting of end-tidal $O_2$ (PETO$_2$) and $CO_2$ (PETCO$_2$) pressure using the sequential gas delivery method (Slessarev et al., 2007). This method was chosen to maximize steady-state PETCO$_2$-targetting accuracy and stability while minimizing PETO$_2$ confounds during CO$_2$-based CVR measurements (Chen and Pike, 2010b; Halani et al., 2015; Mark et al., 2010; Prisman et al., 2008) and has demonstrated significant advantages over alternative methods (Mark et al., 2010; Tancredi and Hoge, 2013). We used this setup to achieve precise manipulations of basal PETCO$_2$ in each subject. Specifically, in addition to each subject’s natural baseline (normocapnia), we also induced hypercapnic and hypocapnic baselines, which are both separated from the normocapnic baseline by 4 mmHg CO$_2$.

2.3.1 Vascular manipulation for CVR measurement

For CVR measurement, PETCO$_2$ was sinusoidally modulated (Blockley et al., 2011) during the dual-echo pCASL scans at each baseline PETCO$_2$ with a period of 120 s; 3 periods of sinusoidal PETCO$_2$ variations were induced with a baseline-to-peak amplitude of ±4 mmHg, following a 1-minute baseline. This sinusoidal manipulation was applied at the 3 vascular-tension levels described earlier. The ordering of the different baselines was randomized to minimize biases. These mild PETCO$_2$ changes result in slight changes in the subject’s vascular tension without noticeable changes in cerebral oxidative metabolism, a potential confound for CO$_2$-based vascular assessment (Chen and Pike, 2010a). The experimental protocol is illustrated in detail in Figure 2.1.
Figure 2.1: Experimental design. (a) Resting-state fMRI scans were carried out at three different basal capnic states, namely, hypercapnia, normocapnia (subject’s natural baseline) and hypocapnia. The same capnic modulations were applied during measurements of cerebrovascular reactivity (CVR), seen in (b). During the CVR scans, a vasodilatory-vasoconstrictive vascular stimulus, in the form of sinusoidally modulated CO$_2$ (c), was administered over 6 minutes, spanning 3 periods of the sinusoid.

2.3.2 Vascular manipulation for rs-fcMRI

Likewise, rs-fMRI metrics including functional connectivity (fcMRI) were measured at each vascular-tension level (hyper-, hypo-, and normo-capnia). The hypo- and hypercapnic levels were also separated from normocapnia by 4 mmHg CO$_2$, matching those of the CVR measurements. The rs-fMRI scans were performed immediately before the CVR scans, and the match between the rs-fMRI and CVR capnic levels was closely monitored during data acquisition and analysis.
2.4 Data analysis

2.4.1 Preprocessing

Functional images, including the tag and control images in the pCASL data, and T1-weighted anatomical images were separately preprocessed using SPM8 (Wellcome Trust Centre for Neuroimaging, London, UK, (Friston et al., 1994)). The first four time frames were discarded to ensure MR steady state. Preprocessing for functional images included retrospective head motion correction, slice-timing correction using sinc interpolation, spatial transformation into a Montreal Neurological Institute (MNI152) space, and spatial smoothing with a 6-mm full-width at half-maximum (FWHM) Gaussian kernel. We band-pass filtered the rs-fMRI data to the 0.008 – 0.09Hz range. Note that due to our high sampling rate (TR = 0.38 s), we were able to directly filter out the cardiac and respiratory frequencies.

Anatomical images were co-registered with their corresponding realigned functional data and segmented into gray and white matter tissue probability maps using unified segmentation (Ashburner and Friston, 2005). Exploiting this anatomical information, we assume that the physiological noise contribution arising from low-frequency cardiac pulsation and respiration is globally distributed, and that the white matter and cerebrospinal fluid (CSF) could serve as noise regions-of-interest (ROI). The first 3 principal components derived from the signal in the noise ROIs were then removed by projection onto the orthogonal complement of the range space of the noise regressors.

![Figure 2.2: Sample delineation procedure for region of interest (ROI)](image)

**Figure 2.2: Sample delineation procedure for region of interest (ROI)** for a typical subject. The ROI used in the analyses is derived from the intersection of (1) the motor network, (2) the finger-tapping functional scout and (3) the anatomical segmentation of Brodmann’s Area 4.
2.4.2 Region-of-interest definition

We focused our analysis on the motor network for its simplicity, specifically localizing on the hand area of the network to avoid CO\textsubscript{2}-related confounds. Furthermore, we customized the region of interest (ROI) for each subject. A sample illustration of the ROI definition is shown in Figure 2.2.

2.4.2.1 Motor-cortex segmentation

Cortical tissue segmentation was performed using the FreeSurfer package (publicly available at: http://surfer.nmr.mgh.harvard.edu). The procedure includes brain extraction (Segonne et al., 2004), transformation into the MNI152 standard space, intensity normalization (Sled et al., 1998), tessellation of the gray matter white matter boundary, automated topology correction (Segonne et al., 2007), and surface deformation following intensity gradients to optimally place the gray/white and gray/CSF borders at the location where the greatest shift in intensity defines the transition to the other tissue class (Fischl and Dale, 2000). We extracted the primary motor cortical parcellation, segmented as Brodmann’s Area 4 using an automated segmentation algorithm (Fischl et al., 2002), dilated by 1 mm in all 3 planes. This motor parcellation would help to confine our ROI to the primary motor region.

2.4.2.2 Motor functional scout

Based on the motor scout, a $t$-map was generated and thresholded at a $p < 0.05$ level of significance (corrected for cluster size). This region was then overlapped with the FreeSurfer segmentation of primary motor cortex customized to each subject’s native space in order to remove potential false activations elsewhere in the cortex. The conjunction between the two was then overlapped with the functional connectivity map obtained at normocapnic baseline, and thresholded to retain only positive correlation values. The same ROI is then used for all three vascular conditions, as we wished to avoid vascular-condition related ROI-size variations, and aimed to simply compare the strengths of network connectivity.

2.4.3 Cerebrovascular reactivity

For the CVR calculation, the BOLD signal is calculated by averaging consecutive tag and control images from the second echo of the pCASL data. First, the CO\textsubscript{2}-response delay in BOLD and CBF time courses were taken into account at each voxel. This delay was estimated from the
phase difference between the BOLD or CBF time courses and that of PETCO₂, enabling the alignment of the fMRI and PETCO₂ time series for maximal correlation. For this alignment, both PETCO₂ and fMRI data were upsampled to a common higher frequency, such that delays that are shorter than TR could be measured. Following the alignment, the PETCO₂ was interpolated to the pCASL TR and detrended at half the total duration of the respiratory paradigm. Outlier removal was performed based on Cook’s distance from the initial linear model fit and the points with the Cook’s distance greater than 4/N is discarded, where N is the number of time points. Subsequently, a final linear model is fitted to the values and CVR is defined as the slope of the linear model. The spatially specific CVR was thresholded at 0 to minimize biases introduced by noise. Additionally, we excluded data associated with spiking in PETCO₂ time course.

We computed the regional mean and standard deviation of the CVR estimates across each motor ROI. Specifically, in order to generate representative regional CVR values unbiased by outliers, we first removed outlier voxels from each ROI using the non-parametric algorithm based on the Tukey’s box-plot method (Patterson, 2012; Tukey, 1977); this method suits the present application as it does not assume a normal distribution for the voxel-wise CVR values.

2.4.4 Resting-state measurements

As mentioned earlier, we focused on the motor network for its robustness and simplicity. In terms of functional connectivity, we generated motor rs-fcMRI maps using a seed-based analysis approach. All preprocessed images were transformed into MNI152 space. The denoised and band-pass filtered data were used to compute connectivity in the two hemispheres separately, with the contralateral primary motor cortex segmentation was used as the seed. This approach allowed us to minimize autocorrelation-related biases (Arbabshirani et al., 2014). Connectivity was computed as a regression coefficient using the CONN Toolbox (MIT, publicly available at https://www.nitrc.org/projects/conn/). The two hemispheric connectivity maps were then combined to form bilateral motor rs-fcMRI maps.

Given prior literature on the relationship between CVR and the resting-state fMRI signal fluctuation amplitude, we also computed the amplitude of low-frequency fluctuations (ALFF) on the post-processed data (that matches with the data used in the functional connectivity computations). The ALFF was normalized to the mean BOLD signal intensity.
2.4.5 Statistical analyses

We averaged our voxel-wise CVR and rs-fMRI measures within the aforementioned ROIs, and assessed their within-subject dependence on basal capnic level (i.e. hypo-, normo- and hypercapnia), each representing a different level of vascular tension. The significance of any effects was assessed using repeated-measures ANOVA. We also quantified this vascular-state dependence across subjects using a linear regression model, in which the line fits were based on least-squares minimization, and weighted by variability on both the ordinate and the abscissa.
3 Results

Two subjects were excluded from the analysis due to excessive head motion (consistently beyond 1mm), yielding a total group size of \( N = 16 \). In Figure 3.1 we summarize the PETCO\(_2\) and PETO\(_2\) levels achieved during the three capnic levels. As expected, while the PETCO\(_2\) level is significantly different between capnic conditions, PETO\(_2\) alterations are negligible. In addition, there is no significant difference between average PETCO\(_2\) levels associated with the rs-connectivity and CVR data-acquisitions for similar capnic conditions.

![Figure 3.1: Basal PETCO\(_2\) modulations and corresponding PETO\(_2\) levels.](image)

In addition to the group-wise normocapnia level of 40 mmHg CO\(_2\), the desired basal PETCO\(_2\) modulations of +4 mmHg (hypercapnia) and -4 mmHg (hypocapnia) were achieved, creating matched capnic conditions for the fcMRI and CVR runs. All capnic levels were significantly different from one another (asterisk indicates \( p < 0.0001 \)), with error bars representing standard error. These modulations did not result in significant perturbations in PETO\(_2\).

Group-average CVR and functional connectivity maps in the motor network are shown in Figure 3.2. In Figure 3.3, we examine the vascular-state dependence of individual measures in the motor ROI. One-way repeated-measured ANOVA returned significant baseline-associated differences in CVR estimates (\( p = 0.0047 \)). Furthermore, rs-fcMRI and BOLD signal amplitude (ALFF) measurements were both significantly different across vascular baselines (i.e. hypo-, normo- and hypercapnia) (\( F = 3.03, 6.91 \) and 6.78, respectively, \( p < 0.0001 \) in all cases). ALFF and CVR shared a similar trend of being highest at normocapnia. However, this was not the case with
Figure 3.2: Group-average CVR, functional connectivity and amplitude of low frequency fluctuation (ALFF) maps.

Figure 3.3: Group-average CVR, functional connectivity and ALFF values across different capnic conditions. CVR was highest in normocapnia, reduced in the hypercapnic and hypocapnic baselines. Similar trends are observed in ALFF. However, functional connectivity consistently decreases with increasing PETCO₂. Asterisks mark differences with $p < 0.0001$. 
fcMRI, which peaked in hypocapnia. However, CVR and fcMRI did share the feature of being higher during normocapnia than during hypercapnia.

**Figure 3.4:** The relationship between CVR and functional connectivity measurements. (a) Each filled symbol represents the value from one subject averaged across all vascular conditions. The different coloured symbols represent different subjects (corresponding to the 3 identical symbols to represent 3 vascular conditions for each subject). (b) The connectivity-CVR relationship is detailed for each participant, with the middle point of the line plots corresponding to the normocapnic baseline, and the bolded symbol representing the hypercapnic baseline.

To further investigate the association between CVR and functional connectivity, we plotted the connectivity strength versus CVR for different subjects and capnic conditions (Figure 3.4a, 16 subjects, each with three capnic conditions). To avoid multiple-comparison biases, we averaged all values within each subject, and thus present a fit of inter-condition averages in Figure 3.4 to Figure 3.6, with different colors representing different subjects. The motor-ROI functional connectivity values were found to be significantly associated with CVR (Figure 3.4a), characterized by a slope of 3.45, $r^2 = 0.18$ and $p = 0.047$. However, when subjects were examined across their respective vascular baselines, a substantial inter-subject variability was found (Figure 3.4b).
Figure 3.5: The relationship between connectivity and ALFF. Each filled symbol represents the value from one subject averaged across all vascular conditions. The different coloured symbols represent different subjects (corresponding to the 3 identical symbols to represent 3 vascular conditions per subject). (b) The connectivity-CVR relationship is detailed for each participant, with the middle point of the line plots corresponding to the normocapnic baseline, and the bolded symbol representing the hypercapnic baseline. No significant relationship was found between ALFF and connectivity in the motor ROI.

As mentioned earlier, the inclusion of ALFF in the investigation was driven by the potential mediating role between CVR and fcMRI measures. When all participants and conditions were taken into account, ALFF was not significantly associated with rs-fMRI functional connectivity values in our ROIs (Figure 3.5a). Furthermore, the association between ALFF and CVR failed to meet the significance threshold (Figure 3.6a). High inter-subject variability was observed for these latter regressions as well (Figure 3.5b and Figure 3.6b). We note that we wished to perform the fits in Figure 3.5 and Figure 3.6 using the same subjects included in the fcMRI-CVR fit, thus we did not perform outlier removal in producing these two sets of plots.
**Figure 3.6: The relationship between ALFF and CVR.** Each filled symbol represents the value from one subject averaged across all vascular conditions. The different coloured symbols represent different subjects (corresponding to 3 identical symbols for the 3 vascular conditions per subject). (b) The connectivity-CVR relationship is detailed for each participant, with the middle point of the line plots corresponding to the normocapnic baseline, and the bolded symbol representing the hypercapnic baseline. No significant relationship was found between ALFF and CVR in the motor ROI.
4 Discussion

CVR is commonly thought to be a biomechanical trait (Kastrup et al., 1998, 1997) of the vasculature that is not directly related to functional networks. However, in this work, we have shown that CVR is significantly and positively associated with resting-state functional connectivity measurements across individuals. However, this relationship was highly variable amongst individuals.

4.1 The association between CVR and resting-state BOLD fMRI connectivity

In accordance with our hypothesis, the first key finding of this work is that rs-fMRI functional connectivity is significantly associated with CVR. Given the bipolar (up and down) nature of intrinsic brain activity, we felt that a bipolar CO$_2$ stimulus (instead of the conventional square-wave hypercapnic stimulus) better emulate any potential influence that CVR may have on resting-state fMRI fluctuations in the context of functional connectivity applications.

Furthermore, while there are alternative ways of measuring CVR, in particular using cerebral blood flow (Halani et al., 2015; Tancredi et al., 2012), our choice of gradient-echo BOLD for CVR measurement is also motivated by the desire to assess BOLD-specific fcMRI associations with BOLD-specific CVR. Our results demonstrate that both hypercapnic and hypocapnic states are associated with decreased CVR. This effect is described in detail in our recent work (Halani et al., 2015), and is in agreement with previous results derived from dynographic (Lopez de Pablo et al., 1982), Doppler ultrasound (Battisti-Charbonney et al., 2011; Carrera et al., 2011), and MRI (Sicard et al., 2003).

The basal capnic-level dependences of CVR and fcMRI values differed, indicating that basal capnic condition affects CVR and fcMRI in distinct ways. On the other hand, the two sets of values were significantly positively correlated across the group. Across the group, rs-fMRI functional connectivity measurements also depend significantly on the baseline capnic state, with the hypocapnic baseline associated with the highest connectivity values, and hypercapnic baseline associated with the lowest connectivity. The latter finding is in agreement with early data from Biswal (Biswal et al., 1997).
The most obvious explanation for this finding is outlined in Chapter 1. Specifically, significant basal vasodilation or constriction induced by our experimental design may shift the BOLD-PETCO\textsubscript{2} curve into the sublinear regime, as can be predicted using Equation [1.1] (Hoge et al., 1999). Thus, during the hypercapnic state, for instance, the sensitivity of BOLD signal to neuronal fluctuations may be reduced compared to those at normocapnia. Consequently a proportionately larger fraction of the BOLD signal fluctuations could be attributed to noise and artifacts (Liu, 2013), resulting in lower neurosensitivity and hence reduced ability to detect functional connectivity, as outlined by Biswal et al. (Biswal et al., 1997). It is notable that such a dependence of rs-fMRI connectivity on CVR is observed even in our group of young healthy adults with no known vascular dysfunction. An alternate explanation relates to the temporal features of the hemodynamic response function, and in turn, its implications in fcMRI computations. The hemodynamic response function (HRF) characterizes the link between the BOLD signal and neuronal activity. It is likely that the shape of this HRF is altered by changes in baseline vascular conditions (Halani et al., 2015). For instance, as reported by Halani et al., the HRF delay lengthens at the hypercapnic baseline in a regional-dependent manner. Should this lengthening vary between the left and right motor cortices, it may engender a reduction in correlation-based connectivity. Such a mechanism would be analogous to previously reported caffeine-induced motor connectivity reduction (Rack-Gomer and Liu, 2012), but further experiments are required to quantify this effect.

Overall, our findings quantify, for the first time, the vascular modulation of functional connectivity measurements, specifically due to CVR, even within a relative narrow range of CVR values, taken from healthy young adults. These findings are consistent with recent discoveries of vascular-neural interplays in fcMRI (Bright and Murphy, 2015; Tong et al., 2015). However, the discrepancy between the CVR and fcMRI trends observed in Figure 3.3 points to a secondary mechanism for the observed fcMRI dependence of basal capnic state, as will be described later.

### 4.2 The role of BOLD signal amplitude

The BOLD fluctuation amplitude has been previously found to explain a significant portion of the vascular reactivity variability seen across subjects (Kannurpatti et al., 2014, 2010, 2011). Moreover, the BOLD fluctuation has been found correlated with inter-subject variability of the
network connectivity strength (Di et al., 2013), although the effect was not found in all functional network regions. Thus, given the bridging role of BOLD fluctuation amplitude between fcMRI and CVR measurements, we were compelled to assess the involvement of ALFF in the observed fcMRI-CVR relationship.

As an analogy from task-based BOLD, previous studies reported reduced BOLD signal amplitude in response to evoked task-related activation during hypercapnic states (Stefanovic et al., 2006), and the opposite for hypocapnia (Cohen et al., 2002). Hypercapnia is vasodilatory agent and increases the CBF, which in turn would increase blood oxygenation. An increment in the baseline blood oxygenation was found to result in reduced BOLD responsiveness to neuronal activity. Such a reduction in the sensitivity of BOLD signal to neuronal fluctuation is conceivable. This is analogous to previous task-based findings that have shown that stimulus evoked BOLD signal amplitude reduces during hypercapnia (Stefanovic et al., 2006).

It is interesting to note that while the resting capnic condition affects both ALFF and fcMRI, the group-levels trends shown in Figure 3.3 are different. This discrepancy seems to indicate that ALFF is more closely related to CVR than is fcMRI. However, the ALFF and CVR are not significantly associated in a linear regression in motor ROI. While this finding suggest that the fcMRI-CVR relationship is potentially mediated by ALFF variations, they also demonstrate that ALFF is not a replacement for CVR and cannot be used to correct for CVR-related biases in fcMRI measurements, at least in this motor-network scenario. This is likely an indication that the ALFF encompasses both purely vascular as well as neurovascular fluctuations, and is thus more complex than CVR or task-evoked fMRI responses.

4.3 Potential metabolic involvement

In this work, we modulated PETCO$_2$ while keeping PETO$_2$ constant in awake and free-breathing humans. While we demonstrated a significant role of CVR in the dependence of fcMRI measurement on CO$_2$-mediated vascular tension, there may be other mechanisms underlying this fcMRI variability. Despite our choice of low levels of PETCO$_2$ manipulations, which were based on the premise of global iso-metabolism (Chen and Pike, 2010a; Jain et al., 2011), we recognize that CO$_2$ may have subject-dependent metabolic implications that induce changes in functional connectivity. Indeed, our observation of reduced rs-fcMRI measurements in the hypercapnic state are in agreement with findings by Xu et al. (Xu et al., 2011). This was attributed to reduced
alpha-wave amplitude, consistent with previous findings of reduced multi-unit activity during hypercapnia (Zappe et al., 2008). Similar electroencephalography (Boynton et al., 1996) power reductions in isoflurane-anesthetized rats were reported in the somatosensory cortex (Nasrallah et al., 2015). More recently, multi-spectral rhythms have been found to decrease with increasing PETCO$_2$ in a linear fashion (Driver et al., 2015), prompting further experiments to study the neurovascular implications of CO$_2$.

In this work, we extend the experimental scope of previous studies to the hypocapnic state, and find that the same trend applies, as fcMRI measurements are higher at hypocapnic compared to normocapnic baseline. While previous work on the topic by Xu et al. (Xu et al., 2011) focused on the default-mode network and attributed the reduced connectivity at hypercapnia to arousal effects, we argue that the default-mode network is unique in many ways and may not offer the generalizable insight into biophysical interplays. In that regard, our concordant finding in the motor network brings into focus the possibility that CO$_2$ affects functional connectivity at a global scale, and not purely through arousal or vigilance alterations. Indeed, as our participants underwent similar respiratory exercises for all three capnic conditions (the only variation being actual PETCO$_2$ levels), their levels of arousal is unlikely to vary with capnic state. However, when using EEG alone, Nasrallah et al. observed an increase in bilateral motor connectivity during hypercapnia (Nasrallah et al., 2015), which seems to contradict the fMRI findings -- we attribute this seeming discrepancy to the involvement of CVR. Thus, potential metabolic effect of CO$_2$ may complement the effect of CVR on fcMRI, and may be mediated by the positive relationship between neuronal activity amplitude and BOLD amplitude, and in turn, the relationship between the latter and BOLD sensitivity in the presence of noise. In this regard, it is interesting to note that CVR and neural metabolism likely both contribute to the sensitivity of BOLD functional connectivity to resting CO$_2$.

### 4.4 Inter-subject variability in the CVR dependence on basal CO$_2$

Our original experimental hypothesis was based on the assumption that a deviation from normocapnia, either in the form of hypercapnia or hypocapnia, would result in some degree of reduced vasoreactivity to a bipolar vascular stimulus. Although we observed this trend at a group level, this was not the case for each individual subject. Interestingly, the relationship between
CVR and the underlying vascular tension was highly variable across subjects. This vast inter-subject variability in vascular response is the second main finding of this work, and was reflected in the inter-subject variability in vascular-tension dependence of functional connectivity values. However, as mentioned earlier, this variability may also be attributable to potential metabolic implications of elevated or reduced PETCO₂ (Xu et al., 2011), and its neurovascular consequences.
5 Conclusions and future work

5.1 Potential caveats and future directions

In this work, we opted for a seed-based approach to compute functional connectivity for its simplicity and usefulness in explaining mechanisms (Biswal et al., 1995; A. Puce et al., 1995). However, we see our results relevant to measurements by other mainstay methods such as independent-component analysis (ICA), as ICA sensitivity to functional networks is also dependent on the BOLD signal-to-noise ratio (Beckmann and Smith, 2004).

In addition, while we minimized the time elapsed between our CVR and rs-fMRI scans to the best of our abilities (mean separation between scans = 13 min), the fact that these data were not acquired simultaneously presents a potential source of confound. We attempted to control such a confound by strictly regulating the PETCO$_2$ agreement in the CVR-fcMRI scan pairs, but other time-related neural factors beyond our control may have contributed to the variability in the inter-subject CVR-fcMRI relationship.

Furthermore, in this work, we focus primarily on the influence of CVR on fcMRI measurements. We recognize that, given the potential entanglement of neural and vascular activity (Bright and Murphy, 2015; Tong et al., 2015), a more complete understanding of our observations regarding CVR would require the involvement of neurophysiological or metabolic measurements. Thus, our future work will involve techniques such as whole-brain MR oximetry (Chen and Pike, 2010a; Lu et al., 2012) and electroencephalography to more comprehensively examine the physiological modulators of rs-fMRI functional connectivity. In addition, given the strong significance of the CVR-related bias on fcMRI seen even in a group of young healthy adults, we will examine the implications of CVR on functional connectivity in older adult and adults with vascular risk.

Another direction for extending these results is the investigation of functional connectivity across other networks of the brain. In this work, our decision to investigate only the motor network is driven in part by technical limitations of standard fMRI acquisition technique. Assessing other networks while maintaining the TR as in this work will require the use of accelerated fMRI acquisition techniques, such as simultaneous multi-slice acquisition (Feinberg and Setsompop, 2013).
5.2 Conclusion

Although cerebrovascular reactivity (CVR) is generally considered a biomechanical trait of the vasculature, it has a significant impact on functional connectivity measurements. In this work, we report a strong positive association between CVR and resting-state fMRI functional connectivity in healthy young adults, especially adults with no known vascular or functional impairment. In addition, we quantify, for the first time, the magnitude of this association, which is non-trivial and may have important implications for the sensitivity, reproducibility and interpretability of fcMRI measurements. Furthermore, our work highlights the vast inter-subject variability of the CVR-fcMRI relationship, suggesting the need for more detailed assessment of systemic physiological factors and their impact of rs-fMRI measurements.
References


Patterson, N., 2012. A robust, non-parametric method to identify outliers and improve final yield and quality. Presented at the CS MANTECH.


Appendix A1: Research ethics approval

The study was approved by the research ethics board (REB) of Baycrest. The confirmation of ethics approval is included on the following page.
Notification of REB Continued Approval

Date: November 20, 2013

To: Chen, J., Khatamian, Y., Weekes-Holder, A.

Re: Magnetic Resonance Imaging Method Development (REB# 11-47)

REB Review Type: Annual
REB Initial Approval Date: December 1, 2012
REB Expiry Date: December 1, 2014
Consent Form(s) Currently Approved for Use: ICF (Version #4, September 23, 2013)

The above-named study has received continued approval from the Baycrest Research Ethics Board (REB) until the expiry date noted above. If the study is expected to continue beyond the expiry date, you are responsible for ensuring the study receives re-approval. The REB must also be notified of the completion or termination of this study and a final report provided.

If, during the course of the research, there are any serious adverse events, confidentiality concerns, changes in the approved project, or any new information that must be considered with respect to the project, these should be brought to the immediate attention of the REB. In the event of a privacy breach, you are responsible for reporting the breach to the Baycrest REB and the Baycrest Privacy Office (in accordance with Ontario health privacy legislation – Personal Health Information Protection Act, 2004). Additionally, the Baycrest REB requires reports of inappropriate/unauthorized use of the information. As the Principal Investigator, you are responsible for the ethical conduct of this study.

The Baycrest Research Ethics Board operates in compliance with the Tri-Council Policy Statement, ICH/GCP Guidelines, the Ontario Personal Health Information Protection Act (2004), and Part C. Division 5 of the Food and Drug Regulations of Health Canada.

Sincerely,

[Signature]

Ron Heslegrave, Ph.D.
Chair, Baycrest Research Ethics Board