Quantitative Mapping of Cerebrovascular Reactivity using Resting-state BOLD fMRI: Validation in Healthy Adults

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Abstract

In conventional neuroimaging, cerebrovascular reactivity (CVR) is quantified primarily using the blood-oxygenation level-dependent (BOLD) functional MRI (fMRI) signal, specifically, as the BOLD response to intravascular carbon dioxide (CO₂) modulations, in units of [% BOLD/mmHg]. While this method has achieved wide appeal and clinical translation, the tolerability of CO₂-related tasks amongst patients and the elderly remains a challenge in more routine and large-scale applications. In this work, we propose an improved method to quantify CVR by exploiting intrinsic fluctuations in CO₂ and corresponding changes in the resting-state BOLD signal (rs-qCVR). Our rs-qCVR approach requires simultaneous monitoring of PETCO₂, cardiac pulsation and respiratory volume. In 16 healthy adults, we compare our quantitative CVR estimation technique to the prospective CO₂-targeting based CVR quantification approach (qCVR, the “standard”). We also compare our rs-CVR to non-quantitative alternatives including the resting-state fluctuation amplitude (RSFA), amplitude of low-frequency fluctuation (ALFF) and global-signal regression. When all subjects were pooled, only RSFA and ALFF were significantly associated with qCVR. However, for characterizing regional CVR variations within each subject, only the PETCO₂-based rs-qCVR measure is strongly associated with standard qCVR in 100% of the subjects ($p \leq 0.1$). In contrast, for the more qualitative CVR measures, significant within-subject association with qCVR was only achieved in 50–70% of the subjects. Our work establishes the feasibility of extracting quantitative CVR maps using rs-fMRI, opening the possibility of mapping functional connectivity and qCVR simultaneously.

Keywords

Cerebrovascular reactivity; resting-state fMRI; end-tidal CO₂; respiratory-volume variability; heart-rate variability; deconvolution; hemodynamic response function; non-invasive vascular mapping

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INTRODUCTION

Cerebrovascular reactivity (CVR) is an important indicator of vascular reserve (Ito et al., 2003) and autoregulatory efficiency (Nur et al., 2009). CVR refers to the degree of vasodilatation or constriction in response to a vascular agent or respiratory task. Clinically, impaired CVR has been associated with risk for stroke and transient ischemic attacks (Liu et al., 2012; Zirak et al., 2014). Given similar diagnoses, individuals with CVR impairment have a much higher risk of disabling stroke than those without (Blaser et al., 2002; Bokkers et al., 2011; Kuroda et al., 2004; Mandell et al., 2011; Mandell et al., 2008; Markus and Cullinane, 2001; Schoof et al., 2007; Silvestrini et al., 2000; Tsivgoulis and Alexandrov, 2008). In addition, reduced CVR has also been cited as a marker for lacunar infarction (Birns et al., 2009; Mandell et al., 2011), microbleeding (Birns et al., 2009; Conijn et al., 2012), as well as cortical atrophy (Fierstra et al., 2010) and cognitive decline in individuals at risk of stroke (Hurford et al., 2014; Kovács et al., 2010). Furthermore, CVR is of direct diagnostic value in patient management (Mandell et al., 2011; Mandell et al., 2008). However, conventional forms of CVR measurement are often inappropriate for those with severe disease risk.

CVR is commonly measured from the fMRI response to vasodilating agents. Approaches generally include acetazolamide injection and respiratory manipulations. While acetazolamide injections are invasive and have been associated with adverse and potentially long-lasting side effects (Asghar et al., 2011; Mancino et al., 2011), respiratory manipulations are relatively non-invasive and safe. They induce changes in the subject's end-tidal CO₂ pressure (PETCO₂), a surrogate for intravascular CO₂ (Mark et al., 2010; Poulin et al., 1996). CO₂ is a potent vasodilator, triggering changes in vascular tone through the arterial baroreflex. PETCO₂ changes can be induced through manually adjusted administration of blended gases (Bandettini and Wong, 1997; Cohen et al., 2004; Yezhuvath et al., 2009), end-tidal forcing (Poulin et al., 1996) or more recently, computerized PETCO₂ targeting (Conklin et al., 2011; Han et al., 2011; Mandell et al., 2011; Mandell et al., 2008; Mark et al., 2010; Mikulis et al., 1989; Mutch et al., 2012; Prisman et al., 2008; Slessarev et al., 2007; Spano et al., 2013; Vesely et al., 2001). The latter method provides immediate and robust PETCO₂ changes, and we have used it extensively in our own work (Chen and Pike, 2010a; Chen and Pike, 2010b; Halani et al., 2015). More recently, breath-holding (Murphy et al., 2011) and deep breathing (Bright et al., 2009) have been proposed as alternatives to modulate PETCO₂, although CO₂ manipulation through breathing circuits is still the dominating approach.

The vascular response to CO₂ is well established (Battisti-Charbonney et al., 2011). Typically, CVR is measured as the ratio between the blood-oxygenation level dependent (BOLD) signal change and PETCO₂ change (by virtue of CBF-BOLD coupling). Generally, respiration-challenge based conventional quantitative CVR measurement (qCVR) approaches require lengthy subject preparations and strict subject cooperation, and are particularly challenging in patients (Lu et al., 2011; Magon et al., 2009; Mandell et al., 2008). In addition, the various styles of breath-holding can introduce difficulties in comparing CVR values across populations. Moreover, undesirable perturbations in neuronal

activity (Jain et al., 2011; Xu et al., 2011) due to the sometimes large PETCO₂ changes (Chen and Pike, 2010a) can compromise the accuracy of conventional CVR measures. More concerning are unwanted side effects of conventional CVR tasks, which may be harmful in certain patient types (Karakaya et al., 2006; Laine et al., 1986). Often, patients do not fulfill the inclusion criteria for the existing CVR measurement approach. Based on prior literature, even within those undergoing CVR mapping, as many as 24.3% are unable to complete the respiratory challenges (Spano et al., 2013). These considerations significantly limit the clinical applicability of conventional CVR techniques, and are particularly prohibitive to those at risk of disease.

This work is motivated by the need to overcome this pressing challenge, and introduces a novel approach to quantify CVR based solely on resting-state fMRI (rs-fMRI), without the need for invasive agents, breath-holding or inhalation manipulations. The rs-fMRI family of techniques (Biswal et al., 1995; Fox and Greicius, 2010; Hedden et al., 2009; van Dijk et al., 2010) is commonly used to capture intrinsic brain activity. However, the rs-fMRI signal also encompasses substantial non-neural contributions (Biswal and Kannurpatti, 2009; Biswal et al., 2007; Kannurpatti et al., 2008; Tong and Frederick, 2010), notably through intrinsic physiological processes. These non-neural components constitute the majority of the rs-fMRI signal variability. While these indirect contributions are commonly discarded, we propose to exploit the vascular nature of physiological rs-fMRI as the foundation for extracting quantitative CVR entirely using rs-fMRI (rs-qCVR). In this context, the term “quantitative” will be used to represent CVR values with units of [% BOLD/mmHg CO₂].

The vascular nature of the rs-fMRI signal is well described in a recent review (Liu, 2013). Specifically, the potential of rs-fMRI and PETCO₂ to offer CVR information has been suggested in a number of previous works (Kannurpatti et al., 2014; Kannurpatti et al., 2010, 2011; Wise et al., 2004). In particular, Wise *et al.* illustrated, for the first time, the strong influence of the rs-fMRI signal by instantaneous fluctuations in PETCO₂ (Wise et al., 2004). In our recent work, building on the biophysical model proposed by Chang et al. (Chang et al., 2009a), we modeled the effects of spontaneous PETCO₂ fluctuations on the rs-fMRI BOLD signal (Golestani et al., 2015). Furthermore, we isolated the PETCO₂ effects from those of other prominent physiological processes including cardiac-rate variability (CRV) and respiratory-volume variability (RVT). It bears mentioning that the rs-fMRI signal is inherently modulated by endogenous fluctuations in PETCO₂ through a similar mechanism to that modulating the fMRI signal in respiratory manipulations. In this study, we (1) capture and exploit the rs-fMRI signal component due to PETCO₂ variability as a means to estimate quantitative CVR (qCVR); (2) compare our resting-state qCVR measurements to those obtained using the prospective CO₂-targeting method (referred to as the *de facto* “standard”); (3) compare the performance of our qCVR method to that of alternative rs-fMRI based non-quantitative CVR methods.

METHODS

Participants

All experiments were performed on 16 healthy young adults (age = 26.5 ± 6.5 years, 7M/9F). Screening questionnaires were used to ensure that participants do not suffer from

diseases or take medications that might compromise brain function or hemodynamics (Chen et al., 2011). All participants were recruited from the Rotman Research Institute Research Participants Database, and were asked to refrain from strenuous exercise for 24 hours and to avoid caffeine/alcohol consumption for 4 hours (Mort and Kruse, 2008) prior to the scans. Written informed consent, in accordance with the Baycrest Research Ethics Board, was obtained from all participants.

Physiological Monitoring

Our experimental set up is outlined in Figure 1. During the rs-fMRI sessions, we recorded heart rate using the scanner's built-in finger oximeter, attached to the left index finger. This would provide estimates of cardiac variability. We also recorded the respiration pattern using a pressure-sensitive respiration belt, connected to the Biopac™ (Biopac Systems, Inc. California). The belt was attached just below the ribcage. The belt recordings are linearly proportional to lung expansion, and reflect respiration. PETCO₂ was passively monitored using a breathing circuit connected to a RespirAct™ system (Thornhill Research, Toronto).

Imaging Protocol

We performed the scans at Baycrest using a Siemens TIM Trio 3 Tesla system (Erlangen, Germany) and a 32-channel matrix head coil. Participants were immobilized using head constraints (including a vacuum bag under the chin to control pitch motion).

1. 3D anatomical scan, MPRAGE, 1 mm³ isotropic resolution, GRAPPA acceleration factor = 2;
2. Resting-state BOLD scan using accelerated scanning for more accurate noise removal, using the simultaneous multi-slice GE-EPI BOLD technique (Setsompop et al., 2011): TR/TE = 380/30 ms, flip angle = 40°, 20 5-mm slices, 64×64 matrix, 4×4×5 mm voxels, axial oblique orientation, 1900 measurements (12 min).
3. Conventional CVR scan: for cross-validation of our proposed method, we estimated BOLD-based CVR using the second echo of a dual-echo pseudo-continuous ASL (pCASL) sequence (TR = 3500 ms, TE_{BOLD} = 25 ms, FA = 90°, 20 slices, 3.44×3.44×6 m³, 120 volumes). The pCASL data was also used in a separate publication concerning CVR measurement (Halani et al., 2015).
 - a. PETCO₂ was sinusoidally modulated (Blockley et al., 2011) during the dual-echo pCASL scans with a period of 120 s; 3 periods of sinusoidal PETCO₂ variations were induced with a baseline-to-peak amplitude of ±4mmHg, following a 1-minute baseline.
 - b. All CO₂ manipulations were achieved by administering mixtures of O₂, CO₂ and medical air delivered using the RespirAct™ breathing circuit (Thornhill Research, Toronto, Canada), designed to provide computerized and independent targeting of end-tidal O₂ (PETO₂) and CO₂ (PETCO₂) pressure using the sequential gas delivery method (Slessarev et al., 2007). This method was chosen to maximize steady-state PETCO₂-targeting accuracy and stability while minimizing

PETCO₂ confounds during CO₂-based CVR measurements (Chen and Pike, 2010b; Halani et al., 2015; Mark et al., 2010; Prisman et al., 2008), and is the most established method clinically (Pillai and Mikulis, 2015).

Accelerated vs. Conventional fMRI Acquisition—In another sub-study with a subset of 8 subjects (5 male, age = 27 ± 5.9 years), we implemented rs-fMRI scans using both accelerated (described earlier) and non-accelerated scanning. The non-accelerated scan parameters are: TR = 2 s, flip angle = 70°, 360 frames (12 min), with other parameters being kept the same as the accelerated BOLD scan. This acquisition method is more widely available on clinical scanners; thus, although it is not most ideal, we wish to assess its feasibility vis-à-vis the accelerated rs-fMRI scan.

Reproducibility Assessment—In a sub-study involving 5 subjects from the current cohort, we performed repeated sessions of conventional and accelerated rs-fMRI acquisition to assess reproducibility of the rs-qCVR estimates associated with each acquisition.

BOLD Data Preprocessing

We first regressed out cardiac and respiratory cycles using the RETROICOR method (Glover et al., 2000). RETROICOR regresses out 2nd-order Fourier series of cardiac and respiratory phase time courses, but its performance can be sensitive to phase-modifying factors such as motion. Given that we sampled our fMRI data at a sufficiently high rate, we were able to test the performance of RETROICOR by observing the frequency spectrum of the rs-fMRI signal. This is shown in Figure S1 of Supplementary Materials, in which we are able to distinguish 3 noise peaks in this figure ($f \sim 0.35$ Hz, 0.7 Hz and 1.2 Hz, related to harmonics of respiration and cardiac noise). The data is representative of our generally healthy young subjects, and shows that while RETROICOR substantially suppresses these noise peaks, it does not fully remove them. Nonetheless, running RETROICOR permitted us to reduce the contribution from irrelevant time-locked signal components, and to isolate on the lower-frequency signal components integral to the BOLD response to CO₂.

Subsequent preprocessing of the rs-fMRI magnitude data included the following steps: (1) retrospective motion correction; (2) slice-timing correction; (3) detrending up to the 3rd order spline (Tanabe et al., 2002); (4) regression of six motion parameters; and (5) spatial smoothing using a Gaussian kernel. These were performed using AFNI (National Institutes of Health, Bethesda, Maryland; <http://afni.nimh.nih.gov/afni>) and the FEAT package in FSL (FMRIB, Oxford University; <http://fsl.fmrib.ox.ac.uk/fsl/fslwiki/FEAT>). Furthermore, we discarded slices with excessive motion (frame-wise displacement ≥ 1 mm), replacing them with interpolated values from neighbouring frames (Huang et al., 2008). Lastly, we normalized the BOLD signal by its temporal mean to arrive at a %BOLD fluctuation time course.

Physiological Modeling in rs-fMRI

Based on the current understanding, the physiological modulation of the rs-fMRI signal can be mainly attributed to fluctuations in CO₂, respiratory volume and heart rate. PETCO₂

fluctuations may result in and from respiration-rate changes (Sin et al., 2012) and heart-rate variations (Brown et al., 2007). A robust relationship between PETCO₂ fluctuations and the rs-fMRI signal was first reported by Wise *et al.* (Wise et al., 2004) and reproduced by Chang *et al.* (Chang and Glover, 2009). However, as PETCO₂'s interactions with respiratory volume and cardiac-pulsation rate were not fully accounted for, the unique modulatory effect of PETCO₂ on the rs-fMRI signal remains undefined. In order to lay the foundation for the isolation and use of PETCO₂ for CVR measurements, we must also account for the relationship with its concurrent physiological signals:

- Respiratory-volume variability (RVT): Birn *et al.* first reported that ongoing changes in breathing pattern impacts the rs-fMRI signal (Birn et al., 2006). Believed to be initiated by random dynamics in the autonomic nervous system (Bruce, 1996), this slow (~0.03 Hz) breath-to-breath volume variation was correlated with the BOLD signal in a pattern similar to that of PETCO₂ (Chang and Glover, 2009), and to BOLD maps associated with breath-holding (Birn et al., 2006), suggesting a partially similar, but not identical, mechanism (Chang and Glover, 2009).
- Cardiac-rate variability (CRV): Variability in heart rate has also been found to strongly modulate the rs-fMRI signal (Chang et al., 2009a; Shmueli et al., 2007), not only near the cerebrospinal fluid and major blood vessels, but throughout the grey matter. It is well established that respiration and cardiac pulsation are related through respiratory sinus arrhythmia, a naturally occurring variation in heart rate during a breathing cycle driven by the parasympathetic nervous system. Breathing rate scales with heart rate (Pitzalis et al., 1997) as well as heart-rate variability (Princi et al., 2006). Interestingly, the effects of RVT and CRV on the rs-fMRI signal were found to mirror each other (Petridou et al., 2009) and partially colocalize (Chang et al., 2009a). At the same time, intravascular CO₂ changes are also known to modulate CRV (Brown et al., 2007).

To further justify our approach, an increase in PETCO₂ can result in an increase in RVT via chemoreceptor regulation (van den Aardweg and Karemaker, 2002), but it can also result from neural control of respiration (McKay et al., 2003). Furthermore, an increase in respiratory volume induces increased dynamic susceptibility effects through elevated chest-motion fluctuation, which is reflected in periodic reductions in the BOLD signal in step with respiratory-volume increases, as shown in (Khatamian et al., 2015). This difference in the mechanisms of RVT and PETCO₂ fluctuations is manifested in their effects on the rs-fMRI BOLD signal, as shown by Chang and Glover (Chang and Glover, 2009), and more recently in our own work (Golestani et al., 2015).

The schematic for our CVR estimation and validation strategy is shown in Figure 2. As mentioned previously, we focus on intravascular CO₂ variations (through PETCO₂) while accounting for effects of respiratory volume variability (RVT) and cardiac rate variability (CRV), which are intrinsically related to respiration. RVT and CRV were computed as described previously (Birn et al., 2006; Chang et al., 2009a). Effectively, RVT and CRV are differences between adjacent peaks and valleys in the respiratory volume and cardiac

pulsation signals, respectively, normalized by the time elapsed between these peaks and valleys.

As demonstrated in our prior work, when we estimate the BOLD response to RVT and CRV while accounting for PETCO₂, the results differ from when the three are not fitted concurrently to the BOLD signal (Golestani et al., 2015). Thus, our data-analysis approach build upon these findings, and only consider the BOLD signal that has had the RVT or CRV contributions removed.

CVR Estimation using Resting-State PETCO₂-rs-qCVR Method

Our proposed methodology is outlined in Figure 2. We first estimated the PETCO₂ response function (HRF_{CO_2}) from the resting-state BOLD signal (Eq. 1). We also estimated the onset time in BOLD response to PETCO₂. Specifically, our algorithm involves the following steps:

1. Discard the first 200 frames of the BOLD signal (for signal stabilization), and bandpass rs-fMRI BOLD signal to between 0.005 and 0.4 Hz;
2. Orthogonalize CRV and RVT traces against the PETCO₂ time course; downsample to match rs-fMRI data;
3. Estimate voxel-wise HRF for residual (orthogonalized) CRV and RVT signals based on a multi-variate deconvolution model (Golestani et al., 2015).
4. At each voxel, convolve the orthogonized CRV and RVT with their respective hemodynamic response functions (HRFs), and regress the result from the preprocessed rs-fMRI data;
5. Estimate the relationship between the resting BOLD signal and the PETCO₂ fluctuations. To accomplish this, we experimented with two approaches, referred to as the “*cross-correlation*” and the “*parametric deconvolution*” methods, as detailed in the following.

Cross-correlation Method—This method is motivated by the use of cross-correlations in used in conventional controlled-CO₂ CVR calculation, based on the assumption that increases in PETCO₂ result in an increase in the BOLD signal (Cohen et al., 2002), and vice versa. First, the PETCO₂ signal and the BOLD signal were cross-correlated to find time lag that maximizes the cross correlation. The BOLD signal was shifted one frame at a time, and was assumed to always lag the PETCO₂ signal (Lindquist et al., 2009). In addition, voxels with lag >100 frames (~40 s) do not conform with physiological restrictions and thus were ignored.

Subsequently, both the BOLD and PETCO₂ signals were truncated to contain only the overlapping segment. At this point, any frames that were more than two standard deviations away from the mean of either one of the signals were discarded as outliers. Finally, the PETCO₂ signal (without convolution with hemodynamic response) was regressed against the rs-fMRI signal using the least squares method, and the slope of this fit was recorded for each voxel as the qCVR estimate. In Figure 3 is shown an example of such a linear regression,

with the slope in units of % BOLD/mmHg. As there were approximately 1700 time points in the preprocessed data, these were binned into groups of 100 samples each in the future for better visualization. The error bars in the figure represent the variability in the BOLD signal across each of these bins.

Physiologically speaking, CVR values should be positive. However, the combination of low vascular response and signal to noise ratio in the white matter could result in negative CVR values in some voxels. In the literature, healthy young adults are not expected to present with negative CVR values during a CO₂ challenge (Leung et al., 2016), except for in the ventricles and other areas occupied by cerebrospinal fluid (Blockley et al., 2011; Ravi et al., 2016). This has been the case in our own work as well (Chen and Pike, 2010b; Halani et al., 2015). Therefore, as a last step, all negative CVR values were clamped to zero.

Parametric Deconvolution Method—In the work by Wise et al. (Wise et al., 2004), a canonical HRF, estimated using transcranial Doppler flowmetry, was applied when estimating the fractional BOLD effect due to PETCO₂. In our previous work, we employed voxel-wise deconvolution within a multi-regression model (Golestani et al., 2015) to estimate the HRF for PETCO₂. Specifically, voxel-wise brain responses to PETCO₂, CRV and RVT were simultaneously estimated as shown in Eq. (1)

$$y = X_{CRF} * CRF + X_{RVT} * RRF + X_{CO_2} * RF_{CO_2} + \varepsilon \quad (1)$$

where y is the normalized rs-fMRI BOLD signal, X s are the normalized physiological signals, and CRF, RRF, and RF_{CO₂} are voxel-wise response functions to CRV, RVT, and PETCO₂ respectively. Each response function was modelled as a Gaussian process, as described in (Chang et al., 2009a), using a squared exponential kernel to model the covariance matrix (with a length scale parameter of 4 seconds and a 40-second response length).

However, as the deconvolution process is highly sensitive to noise, the immediate results of the deconvolution did not always yield physiologically plausible HRF shapes. That is, the voxel-wise HRFs were observed to contain non-physiological oscillations, which is particularly problematic for a voxel-wise CVR estimation that is dependent on the HRF amplitude. To overcome this challenge, we employed a parametric deconvolution approach, and fit our PETCO₂ time courses to BOLD data with the goal of parameterizing a HRF_{CO₂} constructed from variants of a canonical HRF. The details are described in the Appendix. Next, we normalize HRF_{CO₂} such that its sum is unity. Finally, we convolve the PETCO₂ time course with the normalized voxel-wise HRF_{CO₂}, and the result is regressed against the preprocessed BOLD signal at each voxel, and the slope of this regression was taken as the resting state quantitative CVR (rs-qCVR), with the unit of % BOLD/mmHg. A sample regression fit is shown in Figure 4.

CVR Estimation using rs-fMRI–Non-quantitative rs-CVR Methods

Previous evidence has suggested that the rs-fMRI amplitude and the global rs-fMRI signal may both provide qualitative resting CVR estimates (rs-CVR). Thus, we also include the global-signal regression approach and two variants of the rs-fMRI amplitude in our comparison, namely,

- The normalized resting-state fluctuation amplitude (RSFA) – the RSFA was originally computed as the temporal standard deviation of the preprocessed and band-pass filtered BOLD time course (Kannurpatti and Biswal, 2008), and been found to be related to breath-hold CVR previously (Biswal et al., 2007). In this work, in order to better distinguish the RSFA from the ALFF, we adopted the normalized version of RSFA, defined as the standard deviation divided by the temporal mean of the rs-fMRI signal (Liu et al., 2013).
- The amplitude of low-frequency fluctuations (ALFF), computed as the root mean square of the frequency spectrum of the preprocessed and band-pass filtered BOLD time course within the 0.01–0.08 Hz frequency band (Yang et al., 2007). This is similar in nature to the RSFA, except for the fact that there is no voxelwise normalization by the BOLD signal mean, thus allowing the ALFF to take into account regional differences in quantitative BOLD amplitude. We chose ALFF over fractional ALFF (fALFF) in our comparison, since fALFF is the signal power in [0.01, 0.08 Hz] normalized by the power of the whole spectrum (Zou et al., 2008), and the normalization would likely reduce the vascular contribution to the fALFF, thereby compromising its vascular link.
- The global-signal regression coefficient - the global-average gray-matter rs-fMRI signal was regressed against the preprocessed BOLD signal at each voxel, and the slope of this regression taken as a surrogate of CVR (Liu et al., 2015). This approach was chosen as an additional measure because the global rs-fMRI signal has been linked to respiratory and cardiovascular fluctuations (Christen et al., 2015). Moreover, Liu *et al.* (Liu et al., 2015) recently demonstrated strong associations between the global-signal correlation and CVR.

Comparison with CVR Estimated based on CO₂ Challenge (“Standard” qCVR)

For the “standard” quantitative CVR, we computed the BOLD signal by averaging consecutive tag and control images from the second echo of the pCASL data. First, the CO₂-response delay in BOLD time course was 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 fit and the points with the Cook’s distance greater than 4/N is discarded, where N is the number of time points (Bollen and Jackman, 1990). Subsequently, a linear model is fitted to the BOLD (dependent variable) and PETCO₂ (independent

variable) values and CVR is defined as the slope of the linear model. As before, 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.

Validation and Reproducibility Analysis

The rs-CVR and rs-qCVR maps were compared with the standard CVR maps quantitatively. We computed the regional mean and standard deviation of the CVR estimates across each region of interest (ROI). That is, 32 anatomical ROIs were generated by FreeSurfer (Fischl et al., 2002) and transformed into the functional space using FSL FLIRT (Jenkinson et al., 2002). Specifically, the parcellations were generated using probability-driven segmentation (Dale et al., 1999) on the high-resolution anatomical scan acquired in the same session. 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). In addition, 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.

By plotting CVR regional averages of the standard versus resting state CVR estimates, we could assess the feasibility of our methods. As we mapped the CVR values on a per-ROI basis, we had to account for variability in BOLD fMRI signal sensitivity and potential artifacts in different brain regions. Thus, in this validation process, we excluded ROIs that contained less than 50% of non-zero fMRI data (potentially as a result of susceptibility artifacts). Furthermore, a voxel outlier removal step then removed all voxels that contained values that were more than 1.96 standard deviations away from its ROI average. Subsequently, we determine, using linear regression, whether a strong concordance exists between rs-qCVR and standard qCVR measures. By comparing the significance of the linear fit as well as other statistical parameters, we could also compare between our quantitative-CVR against the qualitative methods (RSFA, ALFF and global regression). Statistical significance of the regression is evaluated based on Student's *t*-test. In performing these fits, we used two ways to determine the outlier ROIs:

1. Method-specific outlier removal: in regressing rs-CVR against standard CVR for each method, outlier-ROI 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 (Bollen and Jackman, 1990);
2. Method-independent outlier removal: based on step 1, identify the most frequent common outlier ROIs for all methods, and exclude only those.

Moreover, we assessed the agreement between relative CVR values. That is, we normalized each rs-CVR map and each standard CVR map by its respective global CVR mean, such that

we obtained maps of “local-to-global CVR ratios” that would still reflect within-subject regional variations in CVR. These ratios can be compared across subjects, and can also be compared with similarly normalized standard CVR maps. We assessed the regression coefficient (slope) between relative rs-CVR and relative standard CVR maps as a means to assess their association. Ideally, that slope should approach unity.

In addition, we assessed test-retest reproducibility of rs-fMRI-based methods as applied to accelerated and conventional rsfMRI acquisitions ($N = 5$). Our metrics are the intra-class correlation coefficient (ICC) (Anderson et al., 2011; Chou et al., 2012; Faria et al., 2012; Zuo and Xing, 2014) and coefficient of variation (CV) (Lipp et al., 2015) of the sets of rs-CVR estimates obtained for various brain regions. The ICC is defined as the ratio of inter-subject variance to total variance (inter-subject + inter-session variance), while the CV is computed by dividing the standard deviation of the test-retest CVR estimates by their mean.

To assess the feasibility of estimating HRF_{CO_2} using conventional rs-fMRI acquisitions, we compared the estimates with those based on accelerated rs-fMRI acquisitions using the cosine-similarity index (Kovács et al.). The CSI was computed between conventional and accelerated rs-fMRI based estimates, namely the peaks and estimated delays in the CO_2 response functions, as well as the rs-qCVR estimates themselves.

RESULTS

All 16 subjects were able to complete this study. For each subject, we generated standard CVR estimates from CO_2 manipulations, as well as rs-fMRI-based CVR estimates, both quantitative (based on cross-correlation and parametric-deconvolution rs-qCVR methods) and qualitative (based on RSFA, ALFF and global-regression). The group averages of these maps are shown in Figure 5a, while the maps of a representative subject are shown in Figure 5b. All methods generated CVR maps with qualitative similarity to the standard CVR maps, in that grey matter regions exhibited higher CVR. However, a quantitative assessment of agreement was difficult in this case.

In Figure 6, we show initial quantitative comparisons of all methods to the standard CVR method, through a group-wise examination of CVR values taken across all grey matter voxels. Amongst these voxels, only an average of <3.5% exhibited negative CVR values. At this global level, not all methods demonstrated significant cross-sectional agreement with the standard CVR method, as demonstrated through the p values. Notably, the RSFA demonstrated the most significant association with the standard CVR in this case, whereas the parametric deconvolution method demonstrated the least significant correlation across subjects. However, this plot does not convey CVR estimation accuracy in terms of its within-subject spatial variations.

In Figure 7, we demonstrate, for a representative subject, the degree of within-subject agreement between the rs-fMRI-based and standard CVR methods, with the cortex grouped into a number of bilateral anatomical regions of interest (ROIs, as described in Table 1). In this case, a different distinction amongst the different methods begins to surface, with the quantitative CVR estimates produced by the parametric-deconvolution method

demonstrating the highest degree of spatial agreement with CVR from the standard approach. The ALFF, by its original definition, captures the raw image fluctuations in the BOLD time series, and therefore does not perform as well as methods that use the normalized BOLD signal changes (in terms of % BOLD change).

In this cross-spatial examination, we observed some common trends in terms of problematic regions for rs-fMRI-based CVR estimation (for all methods), predominantly the frontal pole, temporal pole, cuneus, inferior temporal regions. These regions are typically associated with poor signal-to-noise ratios (SNRs), resulting in their exclusion from the assessment of spatial agreement with the standard method. Moreover, upon closer examination, 3 data sets were associated with significant and repetitive motion that strongly affected over 50% of the BOLD frames. They were therefore excluded from the analyses, yielding a total of 13 subjects for the remainder of the analyses.

As summarized in Figure 8a and c, the parametric-deconvolution rs-qCVR method produced the best within-subject agreement with the standard CVR approach, with the most number of subjects demonstrating significant or quasi-significant ($p \leq 0.1$) associations between the rs-fMRI and standard CVR values. This is the case whether examining method-specific or a common set of ROIs, with the details underlying this figure included in Tables 2 and 3, respectively. It is also demonstrated in Tables 2 and 3 that rs-fMRI-based CVR values are typically related to standard quantitative CVR values through a scaling factor (labeled “slope”). This slope, which has no physiological significance, simply allows potential calibration of novel CVR measures against the standard quantitative CVR measurements, and is found to be subject-dependent for all rs-fMRI-based methods. However, as shown in Figure 8b, the slope associated with the global-signal regression approach method appears to be the least variable across subjects, followed by the slope of the parametric-deconvolution rs-qCVR method. We repeated the above assessment based on a common set of anatomical parcellations (Silvestrini et al.) for all methods (Figures 8c & d), and observed similar trends. However, as shown in Table 4, the global regression approach is also the only approach that produced CVR estimates that are significantly associated with motion.

The accuracies of relative CVR values estimated using various rs-fMRI methods is summarized in Table 5. As mentioned earlier, relative CVRs are effectively “local-to-global CVR ratios” that reflect within-subject regional variations in CVR, and that can be compared across subjects. We assessed the regression coefficient (slope) between relative rs-CVR and relative standard CVR maps as a means to assess their association. Ideally, that slope should be 1. As seen in Table 5, the slopes relating relative rs-CVR and relative standard CVR vary widely across methods, with the parametric deconvolution method yielding a slope that is closest to 1, followed by the global-signal regression method.

The results of the reproducibility sub-study are summarized in Figure 9. For both accelerated and conventional rs-fMRI acquisitions, the ICC associated with the parametric-deconvolution exceeded 0.6, indicating reproducibility. In addition, the CV for our method ranges between 0.2 and 0.3, indicating that between-session variability amounts to up to 30% of the mean values. The CV values for parametric deconvolution are rather higher than for the alternative methods, only to be exceeded by those of the global-signal regression

approach. We note that due to the limited number of subjects available in this sub-study, we were not able to examine reproducibility at a more refined spatial scale. However, the comparisons with alternative rs-fMRI methods provide necessary perspective for these values.

DISCUSSION

In this work, we introduce an improved method of extracting quantitative CVR based on rs-fMRI data. This method is not conceptually new, and builds upon previous work assessing the effect of spontaneous CO₂ fluctuations on the rs-fMRI signal (Wise et al., 2004). However, the novel aspects include a more comprehensive and spatially specific modeling of physiological effects, not only those of CO₂, but also of RVT and CRV. The main findings of this work are: (1) it is feasible to estimate quantitative CVR from rs-fMRI data with the aid of passive end-tidal CO₂, respiratory-volume and cardiac pulsation recording; (2) compared to alternative and existing rs-fMRI-based methods, our parametric-deconvolution rs-qCVR method achieved substantially better within-subject agreement with the standard CO₂-challenge based CVR method; (3) our rs-fMRI-based method is deemed reproducible, at least in healthy young adults. Although we employed accelerated rs-fMRI acquisitions for our purposes (based on simultaneous multi-slice imaging), we also provide evidence suggesting that our analysis methodology is feasible on non-accelerated rs-fMRI data sets.

We note that within-subject correlation is driven by regionally varying CVR. Such variations can be greater in some subjects than in others, and can be driven by SNR effects. In general, we find that regions with higher resting blood flow (and hence blood volume) (Chen et al., 2011) are associated with higher BOLD CVR (Halani et al., 2015). Such regions are largely in the parietal, occipital and frontal lobes. This may be related to the larger vascular density in these regions, which would in turn contribute to a larger measurable vascular response to CO₂.

Our rs-fMRI-based CVR offers a number of advantages: (1) it circumvents measurement inconsistencies due to variable subject participation and differences in experimental protocols; (2) instead of relying on a few breathing task episodes, we utilize all spontaneous changes in PETCO₂ in our CVR measurement (Fox and Greicius, 2010), resulting in potentially greater measurement reliability; (3) a resting-state method is completely non-invasive, and safe even for those with severe vascular risk factors; (4) it is much less demanding in terms of instrumentation and ease of implementation. As the need for subject cooperation is minimized, our method is expected to have immediate impact for studying various clinical populations, even during general anesthesia, as occurs during neurosurgery.

Reliability of Resting-state PETCO₂ Associations

Our work is inspired by earlier work by Wise *et al.* to formally associate PETCO₂ fluctuations to rs-fMRI signal changes (Wise et al., 2004). It has since been suggested that the rs-fMRI response to intrinsic PETCO₂ fluctuations is not a reproducible metric (Peng et al., 2013), in that it varied with eyes-open and eyes-closed states, for instance. Furthermore, in a comprehensive reproducibility study (Lipp et al., 2015), Lipp *et al.* showed that CO₂-based rs-CVR was associated with low ICC, thus deemed to not be reproducible.

Nonetheless, the main differences between these previous works and our work are: (1) we took care to first remove overlapping variations between RVT, CRV and PETCO₂; (2) as we employed highly accelerated rs-fMRI acquisitions, we were able to remove frequency bands associated with the primary energy peaks of respiration and cardiac pulsation; (3) we applied a voxel-wise parametric deconvolution model instead of simple linear regression with the PETCO₂ time course. Although demonstrating the impact of these methodological differences is beyond the scope of this work, our ultimate goal was to use “standard” respiratory-challenge based CVR measurements to establish the validity of our rs-fMRI-based quantitative CVR measurements. As our results demonstrate, despite a myriad of physiological variations across rs-fMRI sessions and across subjects, our rs-qCVR measurements, notably using the parametric-deconvolution rs-qCVR approach, demonstrated significant correspondence to standard CVR measurements. Thus, we have demonstrated that parametric-deconvolution rs-qCVR estimates can serve as CVR surrogates within patients that are unable to submit to respiratory challenges or tasks.

Moreover, in our own preliminary reproducibility analysis, we observed that rs-qCVR estimates obtained using the parametric-deconvolution approach are fairly reproducible in our healthy young cohort, using both accelerated and conventional fMRI acquisitions (Figure 9). This sets the stage for further validation and reproducibility-assessment in older subjects and patients. As noted earlier, the limited number of subjects available in this sub-study, we were not able to examine reproducibility at a more refined spatial scale. As a result, we sought to interpret our findings through comparison with the reproducibility of alternative rs-fMRI methods. We are able to conclude that the reproducibility of our method is comparable, and in some cases rather higher than that of all alternative methods. Nonetheless, as reproducibility is not the focus of this paper, we will relegate more comprehensive evaluations to our future work.

In an 8-subject sub-study, we used both accelerated and non-accelerated rs-fMRI acquisitions, and arrived at similar conclusions in regards to CVR reproducibility. The %BOLD response to CO₂ depends on acquisition parameters, primarily the TE but also potentially the TR. While the TE is the main deciding factor in BOLD contrast (and hence %BOLD response), TR determines the available SNR and temporal SNR. At a longer TR, such as used in the conventional rs-fMRI acquisition, would enable full relaxation of the spins and hence higher possible image SNR. In the case of the standard CVR method, a dual-echo pCASL acquisition was used to assess BOLD CVR. The averaging of control- and tag-frames in the ASL time series to derive BOLD contrast is a well-established practice (Wong et al., 1997), but the pCASL TR is substantially longer than the rs-fMRI TRs, resulting in potential biases in the absolute CVR values. This is why CVR maps acquired using different acquisition protocols may not be quantitatively comparable, and that we also explored the value of relative CVR estimates.

CVR Estimation Based on BOLD Fluctuation Amplitude

In this work, we included the ALFF as a test of how well the raw BOLD intensity fluctuations captures CVR variations across brain regions. Results show that the raw BOLD signal is outperformed by the normalized BOLD signal in this regard, as exemplified by the

RSFA. Alternative definitions of the ALFF, such as the fractional ALFF (Zou et al., 2008), were not tested in this study, but may offer improved performance, as is seen in the RSFA.

In our study, while the RSFA did not outrank the alternative methods in terms of within-subject accuracy, it was best at representing whole-brain CVR variations across subjects (Figure 6), in reference to the standard CVR method. This echoes prior findings relating the mean motor-cortex RSFA to breath-hold based CVR (Kannurpatti et al., 2014). Moreover, the current study confirms previous findings (Lipp et al., 2015) of the RSFA being highly robust (indicated by the low CV in Figure 9). This low variability is a significant advantage for using the RSFA, and may have contributed to the more significant global association with standard CVR. However, we also confirm prior findings (Lipp et al., 2015) that the RSFA may not provide the best within-subject spatial representation of CVR. In this regard, it is well known that the frequency band associated with RSFA also contains unknown neuronal signals (Leopold and Maier, 2012), which are not accounted for when using RSFA as a surrogate of CVR. Indeed, our data demonstrates that at an individual-subject level, the correlation between RSFA and qCVR is only moderate. The same logic applies to a related metric, the ALFF. The neuronal and vascular contributions to these rs-fMRI summary metrics are likely subject- and region-dependent, and warrants further study.

CVR Estimation Based on Resting-state Global-signal Regression

Liu *et al.* first proposed that the global rs-fMRI signal reflects spontaneous CO₂ fluctuations, and can be used as a regressor for CVR estimation (Liu et al., 2015)—the maps of regression coefficients demonstrated strong within-subject associations with hypercapnia-based CVR maps as well as test-retest reproducibility. A good within-subject agreement with standard CVR was also found in our study, with the global-signal regression approach ranked second only after the parametric-deconvolution approach. However, our preliminary reproducibility assessment showed that the global-signal regression-based CVR surrogates are much less reproducible and more variable than the alternatives the RSFA and ALFF (Figure 9). A potential reason for this is the uniquely strong relationship between global-regression rs-CVR estimates and frame-wise motion, as shown in Table 4. In addition, the extent to which the global signal truly represents vascular effects is still unclear (Scholvinck et al., 2010), warranting further assessments.

Practical Considerations and Limitations

While our results demonstrated strong associations between rs-fMRI and standard task-based CVR values, these two sets of CVR estimates are not equal, and one may need to be calibrated to more closely agree with the other. This may have resulted from discrepancies in the fMRI imaging protocols used for the two CVR scans, mainly through differences in TE, TR and spatial resolution. These parameters influence the level of BOLD signal change detectable, and it should be noted that quantitative CVR values obtained using different imaging protocols can not be directly compared.

Given this difference, a potential question is, “How to calibrate the resting-state quantitative CVR values?”. Such questions have arisen before—for instance, breath-hold-based qCVR estimates are found to be lower than hypercapnia-based qCVR estimates (Tancredi and

Hoge, 2013), but this fact did not hinder the utility of the breath-hold approach. The same is true for the RSFA approach (Kannurpatti et al., 2014), which we found to not be uniformly associated with standard CVR estimates. We argue the same for our parametric-deconvolution-based rs-qCVR estimates, as in many cases, such as in stroke (Mandell et al., 2011) and Alzheimer's disease (Silvestrini et al., 2006; Yezhuvath et al., 2012), CVR maps are used to depict local abnormalities rather than make statements regarding global differences between individuals. In such cases, it is more crucial that the spatial pattern rather than the absolute value of the CVR estimates be accurate, and therefore, calibration of the rs-fMRI CVR values is not necessary. In fact, each CVR map can be normalized by its global mean without loss of generalization in identifying regional CVR deficits, as has been done previously in similar settings (Sojkova et al., 2008).

However, should a globally uniform CVR deficit be the hypothesis, as is the case in hydrocephalus (Chang et al., 2009b), the calibration issue may become more relevant. The fact that the slopes associating our resting-state CVR with standard CVR values are not uniform (Table 2) may present a challenge, raising questions such as "is this calibration-factor variability large enough to prevent the detection of patient-control differences in CVR?". The solution may lie in further testing of the clinical sensitivity of our new CVR approach. The non-invasive and simple nature of our methodology may lead to numerous opportunities for clinical validation. Such clinical validations, which have yet to be performed, are essential before the method can be applied clinically.

We noted that our technique encountered some challenges under 2 conditions: (1) excessive motion; (2) low SNR. Our technique is ideally implemented in data with little motion, as the PETCO₂-related fluctuations account for no more than 20% of the overall rs-fMRI BOLD signal variance (Golestani et al., 2015). For future applications, we recommend the adoption of more advanced motion-correction strategies in preprocessing (Pruim et al., 2015). The challenge of low SNR was generally encountered in regions more severely affected by susceptibility artifacts, including the prefrontal and temporal-pole regions. These regions are associated with lower BOLD signal reliability in general, and novel acquisition techniques may be required to recuperate signal, for instance by optimizing the slice orientation (Weiskopf et al., 2006) or using spin-echo-based rs-fMRI acquisition (Khatamian and Chen, 2014).

On a related topic, while the voxel-wise parametric-deconvolution method yielded the strongest within-subject agreement with the standard method, it is difficult to perform voxel-wise deconvolution due to limited SNR. This may be why earlier studies assumed a single HRF for the whole brain. In our case, this challenge necessitated the subsequent model fitting to ensure that the resulting HRFs are physiologically plausible. Falling short of estimating the HRF for each voxel, we could estimate a single HRF for each ROI. Doing so would prevent us from generating full CVR maps, but would help stabilize the deconvolution, and thus merits further testing.

As stated earlier, one of the major distinctions between our method and prior CO₂-based resting CVR methods is our attempt to orthogonalize PETCO₂, CRV and RVT with respect to one another as a first step. However, the orthogonalization is limited by the fact that we do

not know the actual time delays linking the three variables, and thus have had to assume delays of zero. In the next stages of development, we will seek out ways to refine the orthogonalization step.

Our previous data suggest that the HRF_{CO_2} estimates based on accelerated and conventional rs-fMRI acquisitions are similar (Golestani et al., 2015). The current results are mainly based on accelerated fMRI acquisitions, driven by our desire to avoid noise aliasing. Nonetheless, we have reason to believe our method is adaptable to conventional rs-fMRI, as evidenced in Figure 10. In fact, in our future work, we will employ conventional acquisition speeds in clinical validation. Such a validation will ensure even wider applicability of our method.

Furthermore, we wish to emphasize that it is not necessary to employ a RespirAct unit for quantification of CVR using rs-fMRI. While we used the RespirAct unit for convenience (considering the validation nature of our experimental setup), any instrumentation that can track end-tidal CO_2 fluctuations would suffice, including simple capnometers, with CO_2 monitored using cannulated CO_2 sampling lines.

Finally, the RSFA approach has been recently suggested to provide a non-constrained non-quantitative surrogate for CVR (Kannurpatti et al., 2014), and has demonstrated sensitivity to aging effects. In this study, we clearly demonstrate that quantitative CVR estimated using our resting-state parametric-deconvolution rs-qCVR approach far outperforms the RSFA approach across the group (100% vs. 70% agreement with standard CVR), with potential reasons discussed earlier in this section. In fact, in the absence of dynamic physiological monitoring (cardiac, respiratory, PETCO_2), the global-signal regression method represents the second-ranking alternative for obtaining vascular information, albeit caution is advised, based on the potential neuronal contributions to the global signal.

CONCLUSIONS

The increasing prevalence of cerebrovascular diseases highlights the importance of regularly monitoring cerebrovascular health in those at risk of disease. One of the most valuable indicators of cerebrovascular health is cerebrovascular reactivity (CVR), but CVR is conventionally only obtainable using invasive vasoactive agents or respiratory challenges, making clinical implementation challenging. In response, this paper introduces a novel method to assess CVR based solely on resting-state fMRI data. Exploiting the effect of intrinsic respiratory variability on the rs-fMRI signal, the proposed method would potentially eliminate the need for vascular agents or task challenges. Thus, this method can be applied even to those who have lost consciousness due to illnesses or sedation. We demonstrate the feasibility of our method in healthy controls through validation with the clinically established prospective CO_2 targeting CVR method. Our method is shown to more accurately reflect within-subject spatial variations in CVR than all other rs-fMRI-based alternatives. This work will open up numerous new opportunities to use resting-state fMRI, facilitating the extension of the versatile technique into routine clinical use.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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References

- Anderson JS, Ferguson MA, Lopez-Larson M, Yurgelun-Todd D. Reproducibility of single-subject functional connectivity measurements. *AJNR Am J Neuroradiol*. 2011; 32:548–555. [PubMed: 21273356]
- Asghar MS, Hansen AE, Pedersen S, Larsson HB, Ashina M. Pharmacological modulation of the BOLD response: a study of acetazolamide and glyceryl trinitrate in humans. *J Magn Reson Imaging*. 2011; 34:921–927. [PubMed: 21769961]
- Bandettini PA, Wong EC. A hypercapnia-based normalization method for improved spatial localization of human brain activation with fMRI. *NMR Biomed*. 1997; 10:197–203. [PubMed: 9430348]
- Battisti-Charbonney A, Fisher J, Duffin J. The cerebrovascular response to carbon dioxide in humans. *J physiology*. 2011; 589:3039–3048.
- Birn RM, Diamond JB, Smith MA, Bandettini PA. Separating respiratory-variation-related fluctuations from neuronal-activity-related fluctuations in fMRI. *NeuroImage*. 2006; 31:1536–1548. [PubMed: 16632379]
- Birns J, Jarosz J, Markus HS, Kalra L. Cerebrovascular reactivity and dynamic autoregulation in ischaemic subcortical white matter disease. *Journal of Neurology, Neurosurgery and Psychiatry*. 2009; 80:1093–1098.
- Biswal B, Yetkin FZ, Haughton VM, Hyde JS. Functional connectivity in the motor cortex of resting human brain using echo-planar. *MRI Magn Reson Med*. 1995; 34:537–541. [PubMed: 8524021]
- Biswal BB, Kannurpatti SS. Resting-state functional connectivity in animal models: modulations by exsanguination. *Methods in Molecular Biology*. 2009; 489:255–274. [PubMed: 18839096]
- Biswal BB, Kannurpatti SS, Rypma B. Hemodynamic scaling of fMRI-BOLD signal: validation of low-frequency spectral amplitude as a scalability factor. *Magnetic Resonance Imaging*. 2007; 25:1358–1369. [PubMed: 17482411]
- Blaser T, Hofmann K, Buerger T, Effenberger O, Wallesch CW, Goertler M. Risk of stroke, transient ischemic attack, and vessel occlusion before endarterectomy in patients with symptomatic severe carotid stenosis. *Stroke*. 2002; 33:1057–1062. [PubMed: 11935061]
- Blockley NP, Driver ID, Francis ST, Fisher JA, Gowland PA. An improved method for acquiring cerebrovascular reactivity maps. *Magn Reson Med*. 2011; 65:1278–1286. [PubMed: 21500256]
- Bokkers RP, van Osch MJ, Klijn CJ, Kappelle LJ, Hendrikse J. Cerebrovascular reactivity within perfusion territories in patients with an internal carotid artery occlusion. *Journal of Neurology, Neurosurgery and Psychiatry*. 2011; 82:1011–1016.
- Bollen, KA., Jackman, RW. *Modern Methods of Data Analysis*. Sage; Newbury Park, CA: 1990. p. 257-291.
- Bright MG, Bulte DP, Jezzard P, Duyn JH. Characterization of regional heterogeneity in cerebrovascular reactivity dynamics using novel hypocapnia task and BOLD fMRI. *NeuroImage*. 2009; 48:166–175. [PubMed: 19450694]
- Brown SJ, Mundel T, Brown JA. Cardiac vagal control and respiratory sinus arrhythmia during hypercapnia in humans. *J Physiol Sci*. 2007; 57:337–342. [PubMed: 17996126]
- Bruce EN. Temporal variations in the pattern of breathing. *J Appl Physiol*. 1996; 80:1079–1087. [PubMed: 8926229]
- Chang C, Cunningham JP, Glover GH. Influence of heart rate on the BOLD signal: the cardiac response function. *NeuroImage*. 2009a; 44:857–869. [PubMed: 18951982]
- Chang C, Glover GH. Relationship between respiration, end-tidal CO₂, and BOLD signals in resting-state fMRI. *NeuroImage*. 2009; 47:1381–1393. [PubMed: 19393322]

- Chang CC, Asada H, Mimura T, Suzuki S. A prospective study of cerebral blood flow and cerebrovascular reactivity to acetazolamide in 162 patients with idiopathic normal-pressure hydrocephalus. *Journal of Neurosurgery*. 2009b; 111:610–617. [PubMed: 19284245]
- Chen JJ, Pike GB. Global cerebral oxidative metabolism during hypercapnia and hypocapnia in humans: implications for BOLD fMRI. *J Cereb Blood Flow Metab*. 2010a; 30:1094–1099. [PubMed: 20372169]
- Chen JJ, Pike GB. MRI measurement of the BOLD-specific flow-volume relationship during hypercapnia and hypocapnia in humans. *NeuroImage*. 2010b; 53:383–391. [PubMed: 20624474]
- Chen JJ, Rosas HD, Salat DH. Age-associated reductions in cerebral blood flow are independent from regional atrophy. *NeuroImage*. 2011; 55:468–478. [PubMed: 21167947]
- Chou YH, Panych LP, Dickey CC, Petrella JR, Chen NK. Investigation of long-term reproducibility of intrinsic connectivity network mapping: a resting-state fMRI study. *AJNR Am J Neuroradiol*. 2012; 33:833–838. [PubMed: 22268094]
- Christen T, Jahanian H, Ni WW, Qiu D, Moseley ME, Zaharchuk G. Noncontrast mapping of arterial delay and functional connectivity using resting-state functional MRI: A study in Moyamoya patients. *J Magn Reson Imaging*. 2015; 41:424–430. [PubMed: 24419985]
- Cohen ER, Rostrup E, Sidaros K, Lund TE, Paulson OB, Ugurbil K, Kim SG. Hypercapnic normalization of BOLD fMRI: comparison across field strengths and pulse sequences. *NeuroImage*. 2004; 23:613–624. [PubMed: 15488411]
- Cohen ER, Ugurbil K, Kim SG. Effect of basal conditions on the magnitude and dynamics of the blood oxygenation level-dependent fMRI response. *J Cereb Blood Flow Metab*. 2002; 22:1042–1053. [PubMed: 12218410]
- Conijn MM, Hoogduin JM, van der Graaf Y, Hendrikse J, Luijten PR, Geerlings MI. Microbleeds, lacunar infarcts, white matter lesions and cerebrovascular reactivity -- a 7 T study. *NeuroImage*. 2012; 59:950–956. [PubMed: 21930217]
- Conklin J, Fierstra J, Crawley AP, Han JS, Poubanc J, Silver FL, Tymianski M, Fisher JA, Mandell DM, Mikulis DJ. Mapping white matter diffusion and cerebrovascular reactivity in carotid occlusive disease. *Neurology*. 2011; 77:431–438. [PubMed: 21775744]
- Dale AM, Fischl B, Sereno MI. Cortical surface-based analysis. I. Segmentation and surface reconstruction. *NeuroImage*. 1999; 9:179–194. [PubMed: 9931268]
- Faria AV, Joel SE, Zhang Y, Oishi K, van Zijl PC, Miller MI, Pekar JJ, Mori S. Atlas-based analysis of resting-state functional connectivity: evaluation for reproducibility and multimodal anatomy-function correlation studies. *Neuroimage*. 2012; 61:613–621. [PubMed: 22498656]
- Fierstra J, Poubanc J, Han JS, Silver F, Tymianski M, Crawley AP, Fisher JA, Mikulis DJ. Steal physiology is spatially associated with cortical thinning. *J Neurol Neurosurg Psych*. 2010; 81:290–293.
- Fischl B, Dale AM. Measuring the thickness of the human cerebral cortex from magnetic resonance images. *PNAS Proc Natl Acad Sci USA*. 2000; 97:11050–11055.
- Fischl B, Salat DH, Busa E, Albert M, Dieterich M, Haselgrove C, van der Kouwe A, Killiany R, Kennedy D, Klaveness S, Montillo A, Makris N, Rosen B, Dale AM. Whole brain segmentation: automated labeling of neuroanatomical structures in the human brain. *Neuron*. 2002; 33:341–355. [PubMed: 11832223]
- Fox MD, Greicius M. Clinical applications of resting state functional connectivity. *Front Syst Neurosci*. 2010; 4:19. [PubMed: 20592951]
- Glover GH, Li TQ, Ress D. Image-based method for retrospective correction of physiological motion effects in fMRI: RETROICOR. *Magn Reson Med*. 2000; 44:162–167. [PubMed: 10893535]
- Golestani AM, Chang C, Kwinta JB, Khatamian YB, Chen JJ. Mapping the end-tidal CO₂ response function in the resting-state BOLD fMRI signal: Spatial specificity, test–retest reliability and effect of fMRI sampling rate. *NeuroImage*. 2015; 104:266–277. [PubMed: 25462695]
- Halani S, Kwinta JB, Golestani AM, Khatamian YB, Chen JJ. Comparing cerebrovascular reactivity measured using BOLD and cerebral blood flow MRI: The effect of basal vascular tension on vasodilatory and vasoconstrictive reactivity. *NeuroImage*. 2015; 110:110–123. [PubMed: 25655446]

- Han JS, Abou-Hamden A, Mandell DM, Poubanc J, Crawley AP, Fisher JA, Mikulis DJ, Tymianski M. Impact of extracranial-intracranial bypass on cerebrovascular reactivity and clinical outcome in patients with symptomatic moyamoya vasculopathy. *Stroke*. 2011; 42:3047–3054. [PubMed: 21903951]
- Hedden T, van Dijk KR, Becker JA, Mehta A, Sperling RA, Johnson KA, Buckner RL. Disruption of functional connectivity in clinically normal older adults harboring amyloid burden. *J Neurosci*. 2009; 29:12686–12694. [PubMed: 19812343]
- Huang J, Francis AP, Carr TH. Studying overt word reading and speech production with event-related fMRI: A method for detecting, assessing, and correcting articulation-induced signal changes and for measuring onset time and duration of articulation. *Brain and Language*. 2008; 104:10–23. [PubMed: 17328946]
- Hurford R, Charidimou A, Fox Z, Cipolotti L, Jager R, Werring DJ. MRI-visible perivascular spaces: relationship to cognition and small vessel disease MRI markers in ischaemic stroke and TIA. *Journal of Neurology, Neurosurgery and Psychiatry*. 2014; 85:522–525.
- Ito H, Kanno I, Ibaraki M, Hatazawa J, Miura S. Changes in human cerebral blood flow and cerebral blood volume during hypercapnia and hypocapnia measured by positron emission tomography. *J Cereb Blood Flow Metab*. 2003; 23:665–670. [PubMed: 12796714]
- Jain V, Langham MC, Floyd TF, Jain G, Magland JF, Wehrli FW. Rapid magnetic resonance measurement of global cerebral metabolic rate of oxygen consumption in humans during rest and hypercapnia. *J Cereb Blood Flow Metab*. 2011; 31:1504–1512.
- Jenkinson M, Bannister P, Brady M, Smith S. Improved optimization for the robust and accurate linear registration and motion correction of brain images. *NeuroImage*. 2002; 17:825–841. [PubMed: 12377157]
- Kannurpatti SS, Biswal BB. Detection and scaling of task-induced fMRI-BOLD response using resting state fluctuations. *NeuroImage*. 2008; 40:1567–1574. [PubMed: 18343159]
- Kannurpatti SS, Biswal BB, Kim YR, Rosen BR. Spatio-temporal characteristics of low-frequency BOLD signal fluctuations in isoflurane-anesthetized rat brain. *NeuroImage*. 2008; 40:1738–1747. [PubMed: 18339559]
- Kannurpatti SS, Motes MA, Biswal BB, Rypma B. Assessment of unconstrained cerebrovascular reactivity marker for large age-range FMRI studies. *PLoS ONE*. 2014; 9:e88751. [PubMed: 24551151]
- Kannurpatti SS, Motes MA, Rypma B, Biswal BB. Neural and vascular variability and the fMRI-BOLD response in normal aging. *Magn Reson Imaging*. 2010; 28:466–476. [PubMed: 20117893]
- Kannurpatti SS, Motes MA, Rypma B, Biswal BB. Increasing measurement accuracy of age-related BOLD signal change: minimizing vascular contributions by resting-state-fluctuation-of-amplitude scaling. *Hum Brain Mapp*. 2011; 32:1125–1140. [PubMed: 20665721]
- Karakaya O, Ozdemir N, Kaymaz C, Barutcu I. Dramatic decrease in the pulmonary artery systolic pressure and disappearance of the interatrial shunt with sildenafil treatment in a patient with primary pulmonary hypertension with atrial septal aneurysm and a severe right to left shunt through the patent foramen ovale. *International Journal of Cardiology*. 2006; 110:97–99. [PubMed: 16507322]
- Khatamian, Y., Faraji-Dana, Z., Chen, JJ. *Org Hum Brain Mapp*. Honolulu: 2015. Significance and correction of respiratory off-resonance effects in fMRI - A phantom study; p. 1651
- Khatamian, YB., Chen, JJ. *Intl Soc Magn Reson Med*, Milan. 2014. Respiratory volume over time effects in resting-state gradient-echo and spin-echo EPI BOLD; p. 2998
- Kovács KR, Szekeres CC, Bajkó Z, Csapó K, Molnár S, Olah L, Csiba L. Cerebro- and cardiovascular reactivity and neuropsychological performance in hypertensive patients. *J Neurologica Sci*. 2010; 299:120–125.
- Kuroda S, Shiga T, Ishikawa T, Houkin K, Narita T, Katoh C, Tamaki N, Iwasaki Y. Reduced blood flow and preserved vasoreactivity characterize oxygen hypometabolism due to incomplete infarction in occlusive carotid artery diseases. *J Nucl Med*. 2004; 45:943–949. [PubMed: 15181128]
- Laine JF, Slama M, Petitpretz P, Girard P, Motté G. Danger of vasodilator therapy for pulmonary hypertension in patent foramen ovale. *Chest*. 1986; 89:894–895. [PubMed: 3709258]

- Leopold DA, Maier A. Ongoing physiological processes in the cerebral cortex. *NeuroImage*. 2012; 62:2190–2200. [PubMed: 22040739]
- Leung, J., Kosinski, PD., Croal, PL., Kassner, A. Developmental trajectories of cerebrovascular reactivity in healthy children and young adults assessed with magnetic resonance imaging. *J Physiol*. 2016. Epub ahead of print, February 2016, <http://doi.org/10.1113/JP271056>
- Lindquist MA, Meng Loh J, Atlas LY, Wager TD. Modeling the hemodynamic response function in fMRI: efficiency, bias and mis-modeling. *NeuroImage*. 2009; 45:S187–198. [PubMed: 19084070]
- Lipp I, Murphy K, Caseras X, Wise RG. Agreement and repeatability of vascular reactivity estimates based on a breath-hold task and a resting state scan. *NeuroImage*. 2015; 113:387–396. [PubMed: 25795342]
- Liu P, Hebrank AC, Rodrigue KM, Kennedy KM, Park DC, Lu H. A comparison of physiologic modulators of fMRI signals. *Hum Brain Mapp*. 2013; 34:2078–2088. [PubMed: 22461234]
- Liu, P., Welch, BG., King, D., Li, Y., Pinho, M., Lu, H. *Int Soc Magn Reson Med*. Toronto: 2015. Can resting state fMRI be used to map cerebrovascular reactivity?; p. 50
- Liu TT. Neurovascular factors in resting-state functional MRI. *NeuroImage*. 2013; 80:339–348. [PubMed: 23644003]
- Liu YJ, Huang TY, Lee YH, Juan CJ. The cerebral vasomotor response in varying CO₂ concentrations, as evaluated using cine phase contrast MRI: Flow, volume, and cerebrovascular resistance indices. *Med Phys*. 2012; 39:6534–6541. [PubMed: 23127048]
- Lu H, Xu F, Rodrigue KM, Kennedy KM, Cheng Y, Flicker B, Hebrank AC, Uh J, Park DC. Alterations in cerebral metabolic rate and blood supply across the adult lifespan. *Cereb Cortex*. 2011; 21:1426–1434. [PubMed: 21051551]
- Magon S, Basso G, Farace P, Ricciardi GK, Beltramello A, Sbarbati A. Reproducibility of BOLD signal change induced by breath holding. *NeuroImage*. 2009; 45:702–712. [PubMed: 19211035]
- Mancino R, Varesi C, Cerulli A, Aiello F, Nucci C. Acute bilateral angle-closure glaucoma and choroidal effusion associated with acetazolamide administration after cataract surgery. *Journal of Cataract and Refractive Surgery*. 2011; 37:415–417. [PubMed: 21241929]
- Mandell DM, Han JS, Poubanc J, Crawley AP, Fierstra J, Tymianski M, Fisher JA, Mikulis DJ. Quantitative measurement of cerebrovascular reactivity by blood oxygen level-dependent MR imaging in patients with intracranial stenosis: preoperative cerebrovascular reactivity predicts the effect of extracranial-intracranial bypass surgery. *AJNR American Journal of Neuroradiology*. 2011; 32:721–727. [PubMed: 21436343]
- Mandell DM, Han JS, Poubanc J, Crawley AP, Stainsby JA, Fisher JA, Mikulis DJ. Mapping cerebrovascular reactivity using blood oxygen level-dependent MRI in Patients with arterial stenotic disease: comparison with arterial spin labeling MRI. *Stroke*. 2008; 39:2021–2028. [PubMed: 18451352]
- Mark CI, Slessarev M, Ito S, Han J, Fisher JA, Pike GB. Precise control of end-tidal carbon dioxide and oxygen improves BOLD and ASL cerebrovascular reactivity measures. *Magn Reson Med*. 2010; 64:749–756. [PubMed: 20648687]
- Markus H, Cullinane M. Severely impaired cerebrovascular reactivity predicts stroke and TIA risk in patients with carotid artery stenosis and occlusion. *Brain*. 2001; 124:457–467. [PubMed: 11222446]
- McKay LC, Evans KC, Frackowiak RSJ, Corfield DR. Neural correlates of voluntary breathing in humans. *J Appl Physiol*. 2003; 95:1170–1178. [PubMed: 12754178]
- Mikulis DJ, Chisin R, Wismer GL, Buxton RB, Weber AL, Davis KR, Rosen B. Phase-contrast imaging of the parotid region. *AJNR American Journal of Neuroradiology*. 1989; 10:157–164. [PubMed: 2492717]
- Mort JR, Kruse HR. Timing of blood pressure measurement related to caffeine consumption. *Annals of Pharmacotherapy*. 2008; 42:105–110. [PubMed: 18094346]
- Murphy K, Harris AD, Wise RG. Robustly measuring vascular reactivity differences with breath-hold: Normalizing stimulus-evoked and resting state BOLD fMRI data. *NeuroImage*. 2011; 54:369–379. [PubMed: 20682354]

- Mutch WAC, Mandell DM, Fisher JA, Mikulis DJ, Crawley AP, Pucci O, Duffin J. Approaches to Brain Stress Testing: BOLD Magnetic Resonance Imaging with Computer-Controlled Delivery of Carbon Dioxide. *PLoS ONE*. 2012; 7:e47443. [PubMed: 23139743]
- Nur E, Kim YS, Truijen J, van Beers EJ, Davis SC, Brandjes DP, Biemond BJ, van Lieshout JJ. Cerebrovascular reserve capacity is impaired in patients with sickle cell disease. *Blood*. 2009; 114:3473–3478. [PubMed: 19700663]
- Patterson, N. A robust, non-parametric method to identify outliers and improve final yield and quality. CS MANTECH; Boston: 2012.
- Peng T, Niazy R, Payne SJ, Wise RG. The effects of respiratory CO₂ fluctuations in the resting-state BOLD signal differ between eyes open and eyes closed. *Magnetic Resonance Imaging*. 2013; 31:336–345. [PubMed: 22921940]
- Petridou N, Schäfer A, Gowland P, Bowtell R. Phase vs. magnitude information in functional magnetic resonance imaging time series: toward understanding the noise. *Magn Reson Imaging*. 2009; 27:1046–1057. [PubMed: 19369024]
- Pillai JJ, Mikulis DJ. Cerebrovascular reactivity mapping: an evolving standard for clinical functional imaging. *AJNR American Journal of Neuroradiology*. 2015; 36:7–13. [PubMed: 24788129]
- Pitzalis MV, Mastropasqua F, Massari F, Passantino A, Forleo C, Luzzi G, Totaro P, De Nicolò M, Rizzon P. Dependency of premature ventricular contractions on heart rate. *American Heart Journal*. 1997; 133:153–161. [PubMed: 9023160]
- Poulin MJ, Liang PJ, Robbins PA. Dynamics of the cerebral blood flow response to step changes in end-tidal PCO₂ and PO₂ in humans. *J Applied Physiol*. 1996; 81:1084–1095. [PubMed: 8889738]
- Princi T, Accardo A, Peterec D. Linear and non-linear assessment of heart rate variability in congenital central hypoventilation syndrome. *Biomedical Sciences Instrumentation*. 2006; 42:434–439. [PubMed: 16817647]
- Prisman E, Slessarev M, Han J, Poublanc J, Mardimae A, Crawley A, Fisher J, Mikulis D. Comparison of the effects of independently-controlled end-tidal PCO₂ and PO₂ on blood oxygen level-dependent (BOLD) fMRI. *J Magn Reson Imaging*. 2008; 27:185–191. [PubMed: 18050321]
- Pruim RHR, Mennes M, van Rooij D, Llera A, Buitelaar JK, Beckmann CF. ICA-AROMA: A robust ICA-based strategy for removing motion artifacts from fMRI data. *NeuroImage*. 2015; 112:267–277. [PubMed: 25770991]
- Ravi H, Thomas BP, Peng SL, Liu H, Lu H. On the optimization of imaging protocol for the mapping of cerebrovascular reactivity. *J Magn Reson Imaging*. 2016; 43:661–668. [PubMed: 26268541]
- Scholvinck ML, Maier A, Ye FQ, Duyn JH, Leopold DA. Neural basis of global resting-state fMRI activity. *Proc Natl Acad Sci U S A*. 2010; 107:10238–10243. [PubMed: 20439733]
- Schoof J, Lubahn W, Baeumer M, Kross R, Wallesch CW, Kozian A, Huth C, Goertler M. Impaired cerebral autoregulation distal to carotid stenosis/occlusion is associated with increased risk of stroke at cardiac surgery with cardiopulmonary bypass. *Journal of Thoracic and Cardiovascular Surgery*. 2007; 134:690–696. [PubMed: 17723819]
- Segonne F, Dale AM, Busa E, Glessner M, Salat D, Hahn HK, Fischl B. A hybrid approach to the skull stripping problem in MRI. *NeuroImage*. 2004; 22:1060–1075. [PubMed: 15219578]
- Segonne F, Pacheco J, Fischl B. Geometrically accurate topology-correction of cortical surfaces using nonseparating loops. *IEEE Trans Med Imag*. 2007; 26:518–529.
- Setsompop K, Gagoski BA, Polimeni JR, Witzel T, Wedeen VJ, Wald LL. Blipped-controlled aliasing in parallel imaging for simultaneous multislice echo planar imaging with reduced g-factor penalty. *Magn Reson Med*. 2011; doi: 10.1002/mrm.23097
- Shmueli K, Gelderen PV, de Zwart JA, Horovitz SG, Fukunaga M, Jansma JM, Duyn JH. Low-frequency fluctuations in the cardiac rate as a source of variance in the resting-state fMRI BOLD signal. *NeuroImage*. 2007; 38:306–320. [PubMed: 17869543]
- Silvestrini M, Pasqualetti P, Baruffaldi R, Bartolini M, Handouk Y, Matteis M. Cerebrovascular reactivity and cognitive decline in patients with Alzheimer disease. *Stroke*. 2006; 37:1010–1015. [PubMed: 16497984]
- Silvestrini M, Vernieri F, Pasqualetti P, Matteis M, Passarelli F, Troisi E, Caltagirone C. Impaired cerebral vasoreactivity and risk of stroke in patients with asymptomatic carotid artery stenosis. *JAMA*. 2000; 283:2122–2127. [PubMed: 10791504]

- Sin PYW, Webber MR, Galletly DC, Tzeng YC. Relationship between cardioventilatory coupling and pulmonary gas exchange. *Clin Physiol Funct Imaging*. 2012; 32:476–480. [PubMed: 23031069]
- Sled JG, Zijdenbos AP, Evans AC. A nonparametric method for automatic correction of intensity nonuniformity in MRI data. *IEEE Transactions on Medical Imaging*. 1998; 17:87–97. [PubMed: 9617910]
- Slessarev M, Han J, Mardimae A, Prisman E, Preiss D, Volgyesi G, Ansel C, Duffin J, Fisher JA. Prospective targeting and control of end-tidal CO₂ and O₂ concentrations. *J Physiol*. 2007; 581:1207–1219. [PubMed: 17446225]
- Sojkova J, Beason-Held L, Zhou Y, An Y, Kraut MA, Ye W, Ferrucci L, Mathis CA, Klunk WE, Wong DF, Resnick SM. Longitudinal cerebral blood flow and amyloid deposition: an emerging pattern? *J Nucl Med*. 2008; 49:1465–1471. [PubMed: 18703614]
- Spano VR, Mandell DM, Poulblanc J, Sam K, Battisti-Charbonney A, Pucci O, Han JS, Crawley AP, Fisher JA, Mikulis DJ. CO₂ Blood Oxygen Level-dependent MR Mapping of Cerebrovascular Reserve in a Clinical Population: Safety, Tolerability, and Technical Feasibility. *Radiology*. 2013; 266:592–598. [PubMed: 23204541]
- Tanabe J, Miller D, Tregellas J, Freedman R, Meyer FG. Comparison of detrending methods for optimal fMRI preprocessing. *NeuroImage*. 2002; 15:902–907. [PubMed: 11906230]
- Tancredi FB, Hoge RD. Comparison of cerebral vascular reactivity measures obtained using breath-holding and CO₂ inhalation. *J Cereb Blood Flow Metab*. 2013; 33:1066–1074. [PubMed: 23571282]
- Tong Y, Frederick BD. Time lag dependent multimodal processing of concurrent fMRI and near-infrared spectroscopy (NIRS) data suggests a global circulatory origin for low-frequency oscillation signals in human brain. *NeuroImage*. 2010; 53:553–564. [PubMed: 20600975]
- Tsivgoulis G, Alexandrov AV. Cerebral hemodynamics in acute stroke: pathophysiology and clinical implications. *J Vasc Interv Neurol*. 2008; 1:65–69. [PubMed: 22518224]
- Tukey, JW. *Exploratory Data Analysis*. Addison-Wesley; 1977.
- van den Aardweg JG, Karemaker JM. Influence of chemoreflexes on respiratory variability in healthy subjects. *American Journal of Respiratory and Critical Care Medicine*. 2002; 165:1041–1047. [PubMed: 11956042]
- van Dijk KR, Hedden T, Venkataraman A, Evans KC, Lazar SW, Buckner RL. Intrinsic functional connectivity as a tool for human connectomics: theory, properties, and optimization. *Journal of Neurophysiology*. 2010; 103:297–321. [PubMed: 19889849]
- Vesely A, Sasano H, Volgyesi G, Somogyi R, Tesler J, Fedorko L, Grynszpan J, Crawley A, Fisher JA, Mikulis D. MRI mapping of cerebrovascular reactivity using square wave changes in end-tidal PCO₂. *Magn Reson Med*. 2001; 45:1011–1013. [PubMed: 11378878]
- Weiskopf N, Hutton C, Josephs O, Deichmann R. Optimal EPI parameters for reduction of susceptibility-induced BOLD sensitivity losses: a whole-brain analysis at 3 T and 1.5 T. *NeuroImage*. 2006; 33:493–504. [PubMed: 16959495]
- Wise RG, Ide K, Poulin MJ, Tracey I. Resting fluctuations in arterial carbon dioxide induce significant low frequency variations in BOLD signal. *NeuroImage*. 2004; 21:1652–1664. [PubMed: 15050588]
- Wong EC, Buxton RB, Frank LR. Implementation of quantitative perfusion imaging techniques for functional brain mapping using pulsed arterial spin labeling. *NMR Biomed*. 1997; 10:237–249. [PubMed: 9430354]
- Xu F, Uh J, Brier MR, Hart JJ, Yezhuvath US, Gu H, Yang Y, HL. The influence of carbon dioxide on brain activity and metabolism in conscious humans. *J Cereb Blood Flow Metab*. 2011; 31:58–67. [PubMed: 20842164]
- Yang H, Long XY, Yang Y, Yan H, Zhu CZ, Zhou XP, Zhang YF, Gong QY. Amplitude of low frequency fluctuation within visual areas revealed by resting-state functional MRI. *NeuroImage*. 2007; 36:144–152. [PubMed: 17434757]
- Yezhuvath US, Lewis-Amezcu K, Varghese R, Xiao G, Lu H. On the assessment of cerebrovascular reactivity using hypercapnia BOLD fMRI. *NMR Biomed*. 2009; 22:779–786. [PubMed: 19388006]

- Yezhuvath US, Uh J, Cheng Y, Martin-Cook K, Weiner M, Diaz-Arrastia R, van Osch M, Lu H. Forebrain-dominant deficit in cerebrovascular reactivity in Alzheimer's disease. *Neurobiology of Aging*. 2012; 33:75–82. [PubMed: 20359779]
- Zirak P, Delgado-Mederos R, Dinia L, Marti-Fabregas J, Durduran T. Microvascular versus macrovascular cerebral vasomotor reactivity in patients with severe internal carotid artery stenosis or occlusion. *Acad Radiol*. 2014; 21:168–174. [PubMed: 24439330]
- Zou QH, Zhu CZ, Yang Y, Zuo XN, Long XY, Cao QJ, Wang YF, Zang YF. An improved approach to detection of amplitude of low-frequency fluctuation (ALFF) for resting-state fMRI: fractional ALFF. *Journal of Neuroscience Methods*. 2008; 172:137–141. [PubMed: 18501969]
- Zuo XN, Xing XX. Test-retest reliabilities of resting-state FMRI measurements in human brain functional connectomics: a systems neuroscience perspective. *Neurosci Biobehav Rev*. 2014; 45:100–118. [PubMed: 24875392]

Highlights

- We propose a novel method to quantify cerebrovascular reactivity (CVR) using resting-state fMRI
- We map CVR using intrinsic CO₂ fluctuations
- We demonstrate good spatial agreement between our method and the CO₂-inhalation method
- We demonstrate reasonable repeatability of our method
- Our method does not require any task and is well suited to large-scale patient studies

Figure 1. Experimental set up

In addition to rs-fMRI data, our proposed CVR quantification methodology also relies on the recording of end-tidal CO₂ (PETCO₂), heart-rate and respiratory-volume traces during the rs-fMRI acquisition. These inputs are processed through our physiological modeling algorithm to produce quantitative CVR values.

Figure 2. Schematic of methodology and validation approach

Validation of the rs-fMRI-based quantitative CVR estimates are the focus of this work, and are shaded in red. The gold-standard measurement step, which serve as reference for the validation, is shaded in blue. Alternative rs-fMRI-based CVR metrics, primarily non-quantitative, are highlighted in orange.

Figure 3. Sample fits—cross-correlation method

A section of the time traces for the BOLD (single voxel), PETCO₂ and the optimally shifted PETCO₂ measurements are shown in (a). A sample linear fit of a single-voxel BOLD time series to the shifted PETCO₂ time course is shown in (b), with the slope of the fit designated as the CVR. The preprocessed data were binned into equally spaced bins of 100 points each, in the interest of visualization. The error bars represent the variability in the BOLD signal across each of these bins.

Figure 4. Sample fits—parametric deconvolution method

A single-voxel BOLD time course (blue) overlaid by the fitted time course resulting from the convolution of PETCO₂ and HRF_{CO2} are shown in (a). The corresponding linear fit is shown (b), and CVR is determined as the slope of the fit. Again, The preprocessed data were binned into equally spaced bins of 100 points each, in the interest of visualization. The error bars represent the variability in the BOLD signal across each of these bins.

Figure 5. CVR maps obtained using various approaches
 (a) Average across all 16 subjects; (b) a sample subject.

Figure 6. The correspondence between rs-fMRI and "gold-standard" CVR estimates, based on global grey-matter averages

Each symbol represents the grey-matter average from one of 16 subjects. All methods examined achieved significant agreement with the standard CVR values, although the strength of the correlations varied. The average from each subject excludes respective outlier parcellations for each method.

Figure 7. The correspondence between rs-fMRI and “gold-standard” CVR estimates, based on within-subject agreement in different brain regions, for a representative subject
 Each symbol represents an ROI average CVR, with the number conveying the ROI definition (refer to Table 1). In this case, as is representative across the entire data set, while significant agreement was achieved using the cross-correlation and parametric-deconvolution methods, it was not the case for RSFA and ALFF.

Figure 8. Summary comparison of different rs-fMRI CVR methods

These results do not include data from the 3 subjects exhibiting pronounced motion. Results based on method-specific outlier ROI removals (Table 2) are shown in (a) and (b), while results associated with an identical ROI set for all methods (Table 3) are shown in (c) and (d). The common ROI set is described in Table 1. As seen in both (a) and (c), the parametric-deconvolution method was the only approach to yield quantitative CVR estimates that were in significant or near-significant agreement with the standard CVR measurements in 100% of the subjects, while the global-signal regression method is ranked second. Moreover, as seen in (b), when examining the scaling factor (slope) that related the rs-fMRI based CVR measurements to standard CVR measurements, the global-signal regression method demonstrated the least variability in scaling factors, followed by our parametric deconvolution method.

Figure 9. Reproducibility of resting-state CVR estimates

In a small group of subjects ($N=5$), we compared test-retest reproducibility of resting-state CVR estimates through the intra-class correlation coefficient (ICC) and coefficient of variation (CV). For both accelerated and conventional rs-fMRI acquisitions, the ICC associated with the parametric-deconvolution exceeded 0.6, indicating reproducibility. In addition, the CV for our method ranges between 0.2 and 0.35, indicating that between-session variability amounts to up to 35% of the mean values. These CV values are rather higher than for the alternative methods, only to be exceeded by those of the global-signal regression approach.

Figure 10. Estimating CVR using accelerated vs. conventional rs-fMRI acquisitions

We compare HRF_{CO_2} estimated from our current multi-slice accelerated rs-fMRI acquisition scheme ($TR = 0.38$ s) to those based on conventional rs-fMRI acquisitions ($TR = 2$ s). (a) The shapes of the whole-brain average HRF_{CO_2} estimates are similar (group average, $N = 8$). The amplitude (b) and time of onset (TTP) (c) of the estimates are also found to be similar across 2 sessions for both conventional and accelerated acquisitions in various brain regions, as quantified through the between-session cosine similarity index (similarity threshold = 0.9, shown in green). Finally, as shown in (d), the cosine similarity between rs-qCVR estimates based on accelerated and conventional acquisitions is moderately high in all regions examined.

Table 1
List of numbered brain regions included in the validation

All grey-matter parcellations are obtained using FreeSurfer on a per-subject basis. The frequent-outlier ROIs are highlighted in white. These ROIs are commonly associated with aberrant CVR estimates, in standard and resting-state CVR maps alike.

ROI#	Definition
1	amygdala
2	hippocampus
3	caudal anterior cingulate
4	caudal middle frontal
5	cuneous
6	entorhinal
7	fusiform
8	inferior parietal
9	inferior temporal
10	isthmus cingulate
11	lateral occipital
12	lateral orbitofrontal
13	lingual
14	medial orbitofrontal
15	middle temporal
16	parahippocampal
17	paracentral
18	parsopercularis
19	parsorbitalis
20	parstriangularis
21	pericalcarine
22	postcentral
23	posterior cingulate
24	precentral
25	precuneus
26	rostral anterior cingulate
27	rostral middle frontal
28	superior frontal
29	superior parietal
30	superior temporal
31	supramarginal
32	frontal pole
33	temporal pole
34	transverse temporal

Table 2

Agreement between rs-fMRI-based CVR and standard CVR measurements within each subject, obtained by fitting CVR values from multiple ROIs to those of the standard CVR maps. The results are based on regression across the group of ROIs included are listed in Table 1, and outlier ROIs were identified for each method and excluded. Cases in which a significant spatial correlation with standard CVR measures are shaded in green, while marginally significant correlations ($p \leq 0.1$) are highlighted in yellow. The p value and correlation coefficient (r) corresponding to each subject's linear fit (slope) are listed. This list does not include the three subjects demonstrating excessive motion.

(a) rs-CVR: cross-correlation method			
Subject #	p	r	Slope
11542	0.728	0.08	25.00
7130	<1e-5	0.67	1.54
7934	<1e-3	0.76	1.20
11494	0.02	0.46	1.61
11672	<1e-5	0.78	0.88
7883	0.12	0.32	4.00
10577	0.22	0.27	5.56
10649	0.002	0.60	1.25
11069	<1e-5	0.67	2.38
11164	0.96	0.02	100.00
11175	0.09	0.35	4.00
11570	0.01	0.51	1.12
10831	0.728	-0.091	-13.70

(b) rs-CVR: parametric deconvolution method			
Subject #	p	r	Slope
11542	0.08	0.36	10.00
7130	<1e-4	0.63	2.38
7934	<1e-5	0.82	1.16
11494	0.02	0.50	3.70
11672	0.00	0.71	1.19
7883	0.01	0.49	4.35
10577	0.04	0.42	3.03
10649	0.01	0.52	1.56
11069	<1e-3	0.61	2.63
11164	0.01	0.55	7.69
11175	0.06	0.38	4.00
11570	0.02	0.45	1.37
10831	0.069	0.385	2.36

(c) RSFA			
Subject #	p	r	Slope
11542	<1e-5	0.78	0.02
7130	0.05	0.38	0.01
7934	0.06	0.38	0.01
11494	0.02	0.43	0.01
11672	0.04	0.41	0.02
7883	0.78	0.06	0.002
10577	0.06	0.38	0.02
10649	0.73	0.07	0.002
11069	<1e-5	0.69	0.02
11164	0.26	0.23	0.005
11175	0.01	0.49	0.008
11570	0.01	0.51	0.01
10831	0.551	0.13	0.007

(d) ALFF			
Subject #	p	r	Slope
11542	0.001	0.61	2.6e5
7130	0.47	0.14	4.2e6
7934	0.08	0.35	1.8e5
11494	0.97	0.008	3.7e3
11672	0.03	0.45	2.6e5
7883	0.21	0.25	8.5e4
10577	0.08	0.36	1.2e5
10649	0.24	-0.25	-6.3e4
11069	0.002	0.56	2.1e5
11164	0.67	0.09	3.4e4
11175	0.005	0.50	1.1e5
11570	0.04	0.39	1.5e5
10831	0.64	0.11	4.8e4

(a) Global-signal Regression			
Subject #	p	r	Slope
11542	0.05	0.38	2.43
7130	0.00	0.56	3.95
7934	0.00	0.58	3.64
11494	0.83	0.04	0.17
11672	0.05	0.43	2.89
7883	0.11	0.31	1.54
10577	0.02	0.46	2.25
10649	0.00	0.63	2.33

(a) Global-signal Regression			
Subject #	p	r	Slope
11069	0.01	0.52	2.97
11164	0.83	0.05	0.29
11175	0.00	0.56	1.90
11570	0.00	0.65	3.06
10831	0.05	0.42	3.04

Table 3

Agreement between rs-fMRI-based CVR and standard CVR measurements within each subject, obtained by fitting CVR values from multiple ROIs to those of the standard CVR map. The results are based on regression across the group of ROIs included are listed in Table 1, and a common set of ROIs were used for all methods. Cases in which a significant spatial correlation with standard CVR measures are shaded in green, while marginally significant correlations ($p \leq 0.1$) are highlighted in yellow. The p value and correlation coefficient (r) corresponding to each subject's linear fit (slope) are listed. This list does not include the three subjects demonstrating excessive motion.

(a) rs-CVR: cross-correlation method			
Subject #	p	r	Slope
11542	0.34	0.04	8.54
7130	<1e-5	0.58	1.82
7934	<1e-4	0.53	1.05
11494	0.03	0.21	1.37
11672	<1e-5	0.46	0.91
7883	0.10	0.12	3.98
10577	0.22	0.08	4.38
10649	<1e-5	0.33	1.32
11069	<1e-4	0.46	2.44
11164	0.32	0.06	10.99
11175	0.06	0.16	4.47
11570	0.03	0.19	1.42
10831	0.59	0.02	-8.98

(b) rs-CVR: parametric deconvolution method			
Subject #	p	r	Slope
11542	0.19	0.07	0.07
7130	0.01	0.26	0.28
7934	<1e-5	0.52	0.92
11494	0.50	0.02	0.10
11672	<1e-5	0.48	0.91
7883	0.02	0.23	0.19
10577	0.03	0.20	0.36
10649	0.05	0.19	0.64
11069	0.01	0.26	0.29
11164	<1e-5	0.49	0.17
11175	0.09	0.14	0.23
11570	0.10	0.10	0.51
10831	0.06	0.15	0.48

(c) RSFA			
Subject #	p	r	Slope
11542	<1e-5	0.54	0.0169
7130	0.01	0.26	0.0097
7934	0.14	0.09	0.0072
11494	0.22	0.07	0.0069
11672	0.15	0.12	0.0101
7883	0.18	0.07	0.0066
10577	0.60	0.01	0.0033
10649	0.70	0.01	0.0016
11069	0.00	0.54	0.0173
11164	0.64	0.01	0.0015
11175	<1e-5	0.43	0.0083
11570	0.07	0.14	0.0068
10831	0.88	0.00	-0.0016

(d) ALFF			
Subject #	p	r	Slope
11542	<1e-5	0.40	2.6e5
7130	0.19	0.07	8.5e4
7934	0.21	0.07	1.4e5
11494	0.72	0.01	2.8e4
11672	0.04	0.20	2.5e5
7883	0.05	0.17	1.6e5
10577	0.05	0.16	1.4e5
10649	0.65	0.01	-2.8e4
11069	0.04	0.17	1.4e5
11164	0.53	0.02	-5.1e4
11175	0.01	0.24	1.1e5
11570	0.01	0.30	1.9e5
10831	0.10	0.13	1.7e5

(b) Global-signal Regression			
Subject #	p	r	Slope
11542	<1e-3	0.29	3.21
7130	<1e-3	0.42	4.73
7934	<1e-3	0.33	3.64
11494	<1e-3	0.36	2.38
11672	0.18	0.10	3.13
7883	0.03	0.19	2.61
10577	<1e-5	0.49	3.60
10649	0.03	0.20	2.10

(b) Global-signal Regression			
Subject #	p	r	Slope
11069	0.15	0.08	1.69
11164	0.96	0.00	-0.07
11175	0.03	0.18	1.24
11570	<1e-5	0.32	2.73
10831	0.11	0.12	2.95

Table 4

The correlation between head-motion parameters and whole-brain mean CVR estimates obtained using different rs-fMRI-based methods. The correlation is represented by the coefficient r , and statistical significance by the p value. Significance is indicated by an asterisk (*).

Method	Cross Correlation	Parametric Deconvolution	RSFA	ALFF	Global-signal Regression
Correlation with mean framewise displacement	$r = -0.01$ $p = 0.97$	$r = -0.14$ $p = 0.65$	$r = 0.26$ $p = 0.38$	$r = 0.28$ $p = 0.33$	$r = 0.04$ $p = 0.89$
Correlation with maximum framewise displacement	$r = 0.20$ $p = 0.49$	$r = -0.01$ $p = 0.98$	$r = 0.44$ $p = 0.11$	$r = 0.44$ $p = 0.11$	$r = 0.81$ $p = 0.0004^*$

Table 5
Relative rsCVR estimates, obtained by normalizing each rsCVR value by the global mean, are compared against relative standard CVR values

The table summarizes the slopes of regressions between the relative rsCVR and relative standard-CVR values, corresponding to each rsCVR method. Ideally, the slope should be as close to 1 as possible.

Subject #	Cross Correlation	Parametric Deconvolution	RSFA	ALFF	Global-signal Regression
11542	0.10	0.57	0.62	0.59	0.49
7130	0.71	0.65	0.31	0.12	0.63
7934	0.54	0.57	0.27	0.38	0.77
11494	0.71	0.64	0.33	0.01	0.45
11672	1.01	1.20	0.41	0.54	0.71
7883	0.37	0.82	0.07	0.30	0.51
10577	0.40	0.66	0.50	0.31	0.66
10649	0.80	0.82	0.07	-0.19	0.69
11069	0.62	0.61	0.75	0.59	0.38
11164	0.02	0.42	0.16	0.07	0.001
11175	0.26	0.24	0.39	0.38	0.33
11570	0.66	0.54	0.42	0.42	0.61
10831	-0.26	0.95	0.22	0.12	0.62
Mean ± STD	0.46±0.40	0.67±0.21	0.35±0.20	0.28±0.22	0.52±0.20