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On the assessment of cerebrovascular reactivity using hypercapnia BOLD MRI

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Abstract

Cerebrovascular reactivity (CVR) reflects the capacity of blood vessels to dilate and is an important marker for brain vascular reserve. It may provide a useful addition to the traditional baseline blood flow measurement when assessing vascular factors in brain disorders. Blood-oxygenation-level-dependent MRI under CO₂ inhalation offers a non-invasive and quantitative means to estimate CVR in humans. In this study, we investigated several important methodological aspects of this technique with the goal of optimizing the experimental and data processing strategies for clinical use. Comparing 4 min of 5% CO₂ inhalation (less comfortable) to a 1 min inhalation (more comfortable) duration, it was found that the CVR values were $0.31 \pm 0.05\%/mmHg$ ($N = 11$) and $0.31 \pm 0.08\%/mmHg$ ($N = 9$), respectively, showing no significant differences between the two breathing paradigms. Therefore, the 1 min paradigm is recommended for future application studies for patient comfort and tolerability. Furthermore, we have found that end-tidal CO₂ recording was useful for accurate quantification of CVR because it provided both timing and amplitude information regarding the input function to the brain vascular system, which can be subject-dependent. Finally, we show that inter-subject variations in CVR are of physiologic origin and affect the whole brain in a similar fashion. Based on this, it is proposed that relative CVR (normalized against the CVR of the whole brain or a reference tissue) may be a more sensitive biomarker than absolute CVR in clinical applications as it minimizes inter-subject variations. With these technological optimizations, CVR mapping may become a useful method for studies of neurological and psychiatric diseases.

Keywords

BOLD; brain; cerebrovascular reactivity; CVR; fMRI; hypercapnia

Introduction

Integrity of cerebral vasculature is important for the normal functioning of the brain. Many brain disorders are associated with vascular abnormality such as acute stroke (1), arteriovenous malformation (2), and arterial stenosis and aneurysms (3). Hypercapnia with brief inhalation of CO₂ presents a potential method for assessing vessel elasticity in the brain (4,5). Inhalation of CO₂ increases the CO₂ content in the lungs, quantified by end-tidal CO₂ (EtCO₂), which is typically at equilibrium with arterial CO₂ partial pressure (6). The increase in arterial CO₂

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pressure will then result in the dilation of the resistance vessels such as small arteries and arterioles, and thereby increase cerebral blood flow (CBF). Such an effect can be measured by blood-oxygenation-level-dependent (BOLD) MRI by mechanisms similar to that of functional MRI (fMRI) (7,8).

Although hypercapnia MRI has previously been used to understand the signal mechanism of BOLD phenomena (7–11), to calibrate fMRI signal (12–15), and to test perfusion techniques, its application in brain disorders has been relatively limited. One potential reason is that the experimental procedure and the data processing strategies have not been standardized, and several aspects of the signal quantification are not optimized or fully understood. For example, for a proof-of-principle study, it may be sufficient to measure the BOLD signal change without measuring or normalizing against the EtCO₂ change. However, this may not be optimal for comparison across subjects/populations because EtCO₂ serves as the ‘input function’ to the cerebral vasculature. The physiologic response of individuals to CO₂ breathing may vary due to variations in breathing rate (BR), breathing depth, lung function, etc., and the normalization to EtCO₂ helps to remove the presence of such inter-subject variations. The EtCO₂ measurement is also useful for comparison with studies mentioned in the literature as different studies may use different CO₂ content (e.g. 3, 5, 7, 8, 10% CO₂) (5,16,17). Also, in designing the hypercapnia experiment, it is important to minimize the duration of hypercapnia (for patient comfort and reducing motion), yet maintaining sufficient physiologic responses (for signal sensitivity). Therefore, it is useful to provide some guidance in terms of the optimal duration of the hypercapnia breathing. Finally, it is important to understand the regional distributions of vascular reactivity and elucidate whether or not inter-subject variation is a global effect or a region-specific effect, as this has important implications in minimizing intra-group variations and improving the sensitivity in detecting between-group differences.

The goal of the present study is to establish an experimental and data processing protocol for hypercapnia MRI that is suitable for clinical applications. We compared two experimental paradigms with a long (less comfortable) and a short (more comfortable) CO₂ breathing duration. The EtCO₂ time courses were recorded and an algorithm was devised to use them as subject-specific regressors in activation detection, thereby improving the detection power and, more importantly, accounting for possible differences in respiratory responses in different individuals. Inter-subject variations in hypercapnia BOLD responses were investigated to determine the region-specificity. A simple normalization approach was proposed to minimize these variations, which may improve the sensitivity of differentiating subject groups in clinical applications.

Materials and Methods

Subjects

Twenty healthy subjects were recruited (11 males and 9 females; age range 19–49 years, 29 ± 8 years) for this study. Informed and written consent was obtained from all subjects and the protocol was approved by the Institutional Review Board of the University of Texas Southwestern Medical Center.

MR imaging

MR experiments were performed on a 3T Philips Achieva MR imaging system (Philips Medical Systems, Best, The Netherlands) using a body coil for transmission and an 8 channel sensitivity encoding (SENSE) head coil for receiving. The subjects were immobilized within the head coil using foam pads.

For the hypercapnia BOLD scan, whole brain imaging was performed using a single-shot gradient echo planar imaging sequence with TR/TE/flip = 3000 ms/30 ms/90°. Twenty-five transverse slices (6 mm thickness) placed along the anterior–posterior commissure line were acquired (FOV 220 × 220 mm², matrix size 80 × 80). Each scan lasted 7 min in which 141 brain volumes were acquired. In addition, a high resolution T1 weighted image was acquired with parameters: TR/TE/Flip = 8.3 ms/3.8 ms/12°, 160 sagittal slices, voxel size 1 × 1 × 1 mm³, field of view 256 × 256 × 160 mm³.

Two CO₂ breathing paradigms were studied. In one of them, the ‘4-min paradigm’, the subject breathed room-air during the first minute of scan, followed by 4 min of 5% CO₂ breathing and final 2 min of air breathing (Fig. 1a). In the other, the ‘1-min paradigm’, the subject breathed room air for 1 min interleaved with 1 min CO₂ breathing, repeated three times (Fig. 1b). Eight subjects (age 30±9 years; 5 males and 3 females) performed both 4- and 1-min paradigms in the same session (with scan order counterbalanced across subjects). In addition, seven subjects (age 27±4 years; 4 males and 3 females) participated in the 4-min paradigm only and five subjects (age 30±10 years; 2 males and 3 females) participated in the 1-min paradigm only.

Before the start of the scan, instructions were given to the subjects regarding the protocol. During the scan, subjects were fitted with a nose clip. Hypercapnia was induced by breathing a gas mixture (5% CO₂, 74% N₂, and 21% O₂) contained within a Douglas bag and delivered to the subject through a two-way non-rebreathing valve and mouthpiece combination (Hans Rudolph, 2600 series, Shawnee, KS). A researcher stayed inside the magnet room throughout the experiment to manually switch the valve to control the breathing of air (either room air or CO₂ bag).

EtCO₂ was recorded continuously during the scan using a capnograph device (Capnogard, Model 1265, Novamatrix Medical Systems, CT). In addition, BR, heart rate (HR), and arterial oxygenation saturation (spO₂) (MEDRAD, Pittsburgh, PA) were also monitored and recorded continuously on a laptop.

Data processing

In neural activation fMRI, the timing of the neuronal activity is often assumed to be the timing of the stimulus paradigm because the propagation of the neural signal (<100 ms) is often much faster than the TR of the sequence. In hypercapnia, however, the timing of the ‘input function’ is often quite different from the paradigm (valve switching) because it would take 5–10 s for the EtCO₂ to show any change. Therefore, EtCO₂ recording is important for both the timing and amplitude of the input function. However, before the EtCO₂ trace can be used as a regressor for analysis of the hypercapnia data, it should be noted that there also exists a lag of the BOLD signal behind EtCO₂, which is the time taken for blood to travel from the pulmonary vascular system to the heart and then to the brain vessels. A typical time for the lag is around 15 s (Fig. 2). To determine the lag on a subject-by-subject basis, a preliminary analysis was performed in which the regions of interest (ROIs) were manually drawn in the thalamus and the averaged BOLD time course within the ROI was correlated with the EtCO₂ trace with various time shifts. The shift at which the EtCO₂ provided the maximal cross-correlation coefficient (CC) with the BOLD time course was defined as the time lag, and the shifted EtCO₂ trace was used as the regressor in the later activation detection (using the general linear model, GLM). We chose to draw the ROIs in the thalamus because it is gray matter (with a relatively high vessel density) and the structure thickness is sufficient for manual drawing (cortical gray matter can be very thin). We assumed that the arterial arrival time to thalamus and to other brain regions are similar and the time difference across brain regions, if any, is less than our MRI temporal resolution (3 s). According to the arrival time measures using arterial spin labeling and dynamic susceptibility contrast (DSC) techniques (18–20), this is valid for the TR used in this study.

Functional imaging time series were processed offline using SPM2 (Wellcome Department of Imaging Neuroscience, London, UK). Pre-processing started with realigning of the functional time series. Structural images were co-registered to the functional image and then segmented into gray matter, white matter, and cerebrospinal fluid (CSF) using a probabilistic algorithm (in statistical parametric mapping, SPM). The individual images were normalized into the Montreal Neurological Institute (MNI) brain template so that a group comparison could be made. A 6mm (FWHM) Gaussian kernel was used for smoothing the resultant time series. The post-processing module provided by SPM2 was used to perform standard voxel-by-voxel GLM analysis on the pre-processed functional time series against the shifted EtCO₂. Following regression, the cerebrovascular reactivity (CVR) with units of %BOLD signal change/mmHg EtCO₂ change was obtained.

ROI analysis on functional data was performed using in-house MATLAB scripts. The MNI template was parcellated into 116 ROIs which were combined to generate nine regional ROIs (Fig. 3a) (21,22). A logic AND operation was then performed between these ROIs and the gray matter masks (defined as having >80% probability to be gray matter) to generate the final ROIs. The averaged BOLD signal time series within each ROI was linearly regressed against the shifted EtCO₂ time course to obtain the CVR value for each ROI (in units of % BOLD signal change/mmHg EtCO₂). Regional CVRs were also normalized by the whole-brain CVR to obtain the relative CVR values.

Results

All the subjects were able to complete the prescribed scan and none reported any discomfort during the task performance. Subjects who participated in the 4-min CO₂ paradigm, reported a slight increase in breathing resistance during the high CO₂ state, whereas most of the subjects in the 1-min CO₂ paradigm could not even distinguish the switch between room air and CO₂ mixture. Table 1 summarizes the physiologic parameters for the normocapnia and hypercapnia conditions for members of both groups. As expected, the most notable change with CO₂ breathing was the EtCO₂ values that increased by 12.1 ± 1.2 mmHg (mean $\pm$ SD, $N = 15$) and 10.6 ± 1.7 mmHg ($N = 13$) for the 4- and 1-min groups, respectively. The EtCO₂ change during the 4-min paradigm was slightly higher ($p = 0.01$), suggesting that the CO₂ accumulation had not plateaued after 1 min of inhalation. However, the difference was relatively small ($\sim 10\%$). HR, BR, and the arterial oxygenation fraction showed a marginal or no change with CO₂ breathing. The BOLD signal lagged behind the EtCO₂ trace (Fig. 2). The optimal time shift in order to obtain the maximum CC was found to be 18.7 ± 3.6 and 15.1 ± 3.6 s for the two groups ($p = 0.01$), respectively. This trend may be related to different lengths of blood containing CO₂ and its dispersion from the lungs to the brain. Similar findings have been noted by Rostrup *et al.* (4). We have also considered the benefit of convolving the EtCO₂ trace with the standard fMRI hemodynamic response function (HRF in SPM) before the shifting and cross-correlation. It was found that the CC using the convolved curve was consistently lower than that using the EtCO₂ trace itself ($p < 0.001$). Therefore, we have used the shifted EtCO₂ trace as the regressor in further analysis.

To study the CVR differences between the 4- and 1-min paradigms, we first compared the results from a subgroup of subjects ($N = 8$), on whom we applied both paradigms in the same session, with half of them undergoing the 4-min paradigm followed by the 1-min paradigm and the other half having the reversed order. While the 4-min *versus* 1-min paired *t*-test showed no difference between the two paradigms ($p = 0.79$), interestingly, a significant difference ($p = 0.03$) was found between the first scan ($0.31 \pm 0.07\%/mmHg$) and the second scan ($0.28 \pm 0.05\%/mmHg$) (regardless of the paradigm). Specifically, the second scan showed lower CVR as compared to the first one. Therefore, we focused on the results of the first scan in further analysis.

Over the whole brain, the gray matter CVR was $0.31 \pm 0.05\%/\text{mmHg}$ ($N = 11$) and $0.31 \pm 0.08\%/\text{mmHg}$ ($N = 9$) for the 4- and 1-min group, respectively, showing no difference between the two breathing paradigms. For the white matter, the values were 0.14 ± 0.03 and $0.15 \pm 0.03\%/\text{mmHg}$, respectively, again showing no group differences. Figure 4 shows the averaged CVR maps for the two groups. Figure 3b plots the gray matter regional CVR values, illustrating regional dependence of the vessel reactivity with cerebellum showing the highest reactivity and the insular and frontal cortices the lowest values.

For all the ROIs studied, large inter-subject variations in CVR were observed, often manifesting a two-fold difference. These widespread values within a group are of concern because they will reduce the ability to detect group differences and/or increase the required sample size for clinical applications. We therefore investigated the characteristics of the inter-subject variations and found that the regional values across subjects were all correlated (intra-class correlation analysis, $p < 0.001$ for both groups). That is, if the subjects had a high CVR in brain region A, they also tend to have a high CVR in brain regions B, C, D, etc. Indeed, we observed that the whole-brain CVR was correlated with all the regional CVR values across subjects (Fig. 5). Therefore, by normalizing the regional CVR to the whole-brain CVR of the same individual, we were able to reduce the spread (defined as the ratio between standard deviation and mean of observations across subjects) by 44.1% ($N = 20$, Table 2). Therefore, for clinical applications in which the expected CVR change is regional but not whole-brain, it is recommended to use the normalized CVR (or relative CVR) from a statistical analysis point of view.

Given the obvious differences in gray/white matter CVR and the common partial volume between these two tissue types, we have also assessed the effect of partial volume and the choice of tissue probability threshold. The tissue segmentation using SPM yielded probabilistic maps of gray/white matters and CSF. That is, for each voxel, three values were given (their sum equals 1), which correspond to the probability of being gray matter, white matter or CSF. We therefore plotted the CVR values as a function of the threshold for each tissue type (Fig. 6). It can be seen in the figure that different thresholds in the definition of tissue types may yield different ROI values and this may explain some of the variations in the reported CVR values in the literature. In choosing the threshold, one needs to consider the fact that if the threshold is too stringent, very few voxels will be included and the signal-to-noise ratio (SNR) of the estimation will be low. The SNR can be improved by reducing the threshold, thereby including a larger number of voxels. However, if the threshold is too low, many voxels with partial-voluming will be included, counteracting the benefit of SNR improvement. It is interesting to note from Fig. 6 that reactivity in CSF voxels appears to be higher than that in gray/white matters. We can explain this pattern by the fact that CSF in subarachnoid space often contains many draining veins which are known to have large BOLD effects. The voxels having the highest CSF probability, on the other hand, are more likely to be in the ventricles, which explains the slight drop in CVR in these voxels.

Discussion

In the present study, we used 5% CO_2 inhalation to assess CVR in the brain and investigated several aspects of experimental design and data processing strategies. On comparing a 4-min inhalation to a 1-min inhalation duration, it was found that the sensitivities are similar and there was no significant bias in the estimation (as shown by CVR in $\%/\text{mmHg}$). Therefore, the 1-min paradigm is recommended for future application studies for patient comfort and tolerability. We found that EtCO_2 recording is useful for the accurate quantification of CVR because it provides both timing and amplitude information regarding the input function to the brain vascular system. Furthermore, we propose that relative CVR (normalized against the CVR of the whole brain or a reference tissue) may be a more sensitive biomarker than absolute CVR in clinical applications as it minimizes inter-subject variations in CVR.

The observed CVR maps were similar to those previously reported in the literature with gray matter revealing greater reactivity than white matter (16). This image contrast was similar to those of CBF (23) and cerebral blood volume (CBV) (24), as they are all vasculature-related parameters and CBF/CBV are known to have large modulation effects on the BOLD signal (7,25). For both groups of subjects, our ROI analysis results revealed a highly significant variability in CVR across brain regions with the observed trend resembling what was reported previously (4,26–28). Kastrup *et al.* (27) have reported a variable vascular response across brain regions with the maximal signal amplitude in the cerebellar and visual cortices, minimal signal change in the frontal cortices along with intermediate values in the sensor-motor cortex. Our results also corroborate with those of Rostrup *et al.* (4) who reported increased hypercapnia-induced BOLD signal changes in the temporal and occipital gray matter as compared to frontal gray matter. Interestingly, using a hyperventilation challenge (i.e. hypocapnia), Posse *et al.* (29) found nearly opposite regional reactivity orders, with maximal reactivity to hypocapnia in the frontal, occipital, and parieto-occipital cortices but least signal changes in the cerebellum. Thus, it appears that the vessel reactivity is ‘directional’ to some degree. That is, when the stimulus is positive, the vessels show large responses to attract more blood, but when the stimulus is negative, the vessels are relatively inert to maintain the present blood supply. Further studies are needed to elucidate the nature of this pattern.

We observed a mean BOLD signal intensity change for the whole-brain gray matter of the order of 3% for both groups (3.7 ± 0.8 and $3.2 \pm 0.8\%$ for the 4- and 1-min group, respectively). Results of a previous BOLD fMRI study indicate an average signal intensity change of $2.78 \pm 0.5\%$ during 5% CO₂ breathing (5). Whole-brain gray matter signal changes of 5.9 ± 1.2 (16) and $4.0 \pm 3.4\%$ (17) were reported for 7 and 8% CO₂ inhalation, respectively. Upon normalizing BOLD signal change to EtCO₂, we observed that the gray matter CVR averaged over the whole brain to be of the order of 0.3% BOLD/mmHg EtCO₂ for both groups. The observed values appear to agree with previously reported CVR measurements using BOLD fMRI: $0.21 \pm 0.06\%$ /mmHg CO₂ (5), $0.25 \pm 0.21\%$ /mmHg CO₂ (17), $0.38 \pm 0.11\%$ /mmHg CO₂ (16), and $0.47 \pm 0.11\%$ /mmHg CO₂ (30). The considerable variation observed in these results could be attributed to factors such as scan parameters, processing strategies, and different partial volume effects.

In the present study, the main results for the 4- and 1-min paradigms were obtained from separate groups of subjects. In a subgroup of subjects ($N = 8$), we also compared the CVR values within the same group. While the paradigm selection did not show a significant effect, the CVR was found to be dependent on the order of the scan, with the second scan showing lower CVR values. Therefore, it appears that there is some ‘adaptation’ in the BOLD response to CO₂ inspiration, which could be attributed to either the blood vessel or the neural tissue. Further investigation is needed to elucidate the nature of this effect.

The observation of a correlation between the regional CVRs across subjects is significant as it implies that for a subject with a higher CVR value in one brain region, the CVR values in other regions also tend to be high. As such, the inter-subject variations in CVR are not simply noise, but appear to reflect different global physiologic statuses in different subjects. The mechanism of this variation is not known but has at least two possibilities: (1) the elasticity of brain vessels across subjects is different, thus resulting in different amounts of increase in blood flow with hypercapnia; (2) the basal physiologic states such as basal blood flow and basal venous oxygenation are different across subjects, which can modulate the BOLD signal amplitude independent of mechanism 1. We have recently shown that the latter mechanism could partly explain the inter-subject variations in neural-activation fMRI signals (31) and it is likely that this mechanism might also be contributing to the hypercapnia BOLD responses. Regardless of the mechanism, these observations suggested a simple method to reduce intra-group variations, by dividing the voxel- or ROI CVR values by the whole brain CVR. As seen from

Table 2, the variation of observed CVR data across subjects reduces considerably upon normalizing to the whole-brain CVR. Hence, a relative CVR measure rather than absolute CVR would be more sensitive in detecting group differences in vascular reactivity as it removes the effects of global CVR variations across subjects. This result is particularly relevant for application to disease populations since minimizing intra-group variations can improve the statistical significance and/or reduce necessary sample sizes. However, we note that when the expected pathologic CVR changes involve the entire brain, such as in studies of aging, absolute CVR would have to be used.

It is important to note that the present study has assumed that inspiration of 5% CO₂ has a minimal effect on the neural activity and brain metabolism. This assumption has been generally used in previous studies involving various challenges such as breath holding (5), CO₂ inspiration (4,5), and injection of acetazolamide (32). However, due to methodological limitations in the direct measurement of neural/metabolic parameters in humans, the validity of this assumption is still under investigation. Existing reports in the literature have shown inconsistent results, with a few studies (33,34) reporting that the cerebral metabolic rate of oxygen (CMRO₂) remains constant with hypercapnia, while other studies found a decrease (35–37) or increase (38,39) in CMRO₂. A recent study by Zappe *et al.* (40) has also shown that neural activity is slightly suppressed with mild hypercapnia. Therefore, further investigation is needed to verify whether or not CO₂ challenge is indeed a ‘vasculature-only’ challenge.

A potential limitation of our study was that we did not independently control or monitor the end-tidal oxygen (EtO₂) changes that accompany EtCO₂ changes during CO₂ inhalation experiments. The subjects tend to increase their tidal volume as a normal response to the CO₂ inhalation. As a result, it is known that EtO₂ will increase in such an experiment (41). A recent study by Prisman *et al.* (30) showed that the BOLD response to O₂ change is 0.006%/mmHg, with a sensitivity 50 times lower than that for CO₂ (0.3%/mmHg according to the present study). Thus, it is likely that the effect of O₂ increase in our CVR estimation was minimal. One possibility for the low O₂ reactivity is that O₂ is transported primarily by hemoglobin in the blood, with a very limited amount dissolved in the plasma. Thus, even though the EtO₂ in the lung increases considerably, the amount of oxygen in the blood shows only a minimal increase, as determined by the oxygen dissociation curve (42). Thus, the brain vasculature may not experience a large oxygen surge and this was verified by our data wherein the arterial oxygenation (sO₂) during CO₂ breathing did not change significantly as compared to the normocapnia period (Table 1). A second limitation was that we did not measure CO₂-induced changes in blood pressure during our experiments, and blood pressure is closely related to the perfusion pressure which is the driving force for blood flow. An increase in blood pressure during hypercapnia can also contribute to the increased blood flow (43,44). Non-invasive and continuous blood pressure monitoring is often achieved by using a real-time feedback-controlled system such as Finapres (45), but we found the motor used in that system was incompatible with the MRI system. The alternative method is the measurement of blood pressure through an arterial line, but that procedure is quite invasive and was determined to be not applicable.

Conclusion

The present study demonstrates that while vascular reactivity is heterogeneous across the brain, it does not appear to be significantly different between the two breathing paradigms tested. Regional CVRs are correlated across brain regions and indicate the presence of a global factor, specific to each person. Our results suggest that the use of relative CVR instead of absolute CVR helps to control the variability of vasodilatory capacities across subjects and may increase the statistical significance in group studies.

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Abbreviations

BOLD	blood-oxygenation-level-dependent
BR	breathing rate
CBF	cerebral blood flow
CBV	cerebral blood volume
CC	cross-correlation coefficient
CSF	cerebrospinal fluid
CVR	cerebrovascular reactivity
DSC	dynamic susceptibility contrast
EtCO₂	end-tidal CO ₂
fMRI	functional MRI
HR	heart rate
HRF	hemodynamic response function
ROI	region of interest
SNR	signal-to-noise ratio
SPM	statistical parametric mapping
spO₂	arterial oxygenation saturation

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Figure 1. Timing details of the CO₂/air breathing paradigm used for (a) the 4-min and (b) the 1-min groups. Arrows show the CO₂ on and off times. fMRI acquisition volume numbers (vol) are also shown.

Figure 2.

Time course of EtCO₂ and BOLD response (from thalamus) averaged across members of the 1-min group. The figure shows the presence of a hemodynamic lag of the BOLD response with respect to the EtCO₂ waveform.

Figure 3.

Regional CVR values. (a) Brain parcellation illustrating the brain regions in the ROI analysis: insular cortex (Ins), frontal cortex (Fro), central sulcus (Cen), limbic cortex (Lim), subcortical gray nuclei (ScG), temporal cortex (Tem), parietal cortex (Par), occipital cortex (Occ), and cerebellum (Cer). (b) Mean cerebrovascular reactivity for ROIs averaged across subjects of 4-min ($N = 11$) and 1-min groups ($N = 9$), respectively. White bars show CVR values for the 4-min group, whereas black bars are used for the 1-min group. The x -axis shows the ROI labels [explained in (a)], arranged in the order of increasing CVR for the 4-min group. Standard error bars are also shown.

Figure 4. Contiguous transverse slices through the CVR maps averaged over all subjects in the (a) 4-min group ($N = 11$) and (b) 1-min group ($N = 9$), respectively.

Figure 5.

Scatter plots between regional CVR and whole brain CVR for all subjects ($N = 20$). The names of individual ROIs are indicated separately for each plot. ScG refers to the subcortical gray nuclei. Subjects from 4- and 1-min groups are labeled with green triangles and red squares, respectively. A strong positive correlation can be seen for all ROIs ($p < 0.01$). This figure is available in color online at www.interscience.wiley.com/journal/nbm

Figure 6.

Average CVR value plotted against the tissue-probability threshold. For each tissue type (gray matter, white matter, and CSF), thresholding was used to select voxels for spatial averaging of the CVR. Standard error bars are also shown. This figure is available in color online at www.interscience.wiley.com/journal/nbm

Table 1
Physiologic parameters during room air and CO₂ breathing

End tidal CO ₂ (mmHg)		Breathing rate (breaths/min)		Heart rate (beats/min)		Arterial oxygen saturation (%)	
Room air	CO ₂	Room air	CO ₂	Room air	CO ₂	Room air	CO ₂
38.0 ± 2.0	50.1 ± 2.2	14.8 ± 3.7	14.4 ± 3.2	73.6 ± 9.6	74.8 ± 9.8	98.7 ± 0.5	98.7 ± 0.4
$p = 8.26 \times 10^{-16}$		$p = 0.27$		$p = 0.11$		$p = 0.42$	
37.5 ± 3.2	48.1 ± 3.0	14.5 ± 4.3	14.3 ± 4.1	74.9 ± 9.4	75.8 ± 10.0	98.5 ± 0.4	98.4 ± 0.6
$p = 4.69 \times 10^{-11}$		$p = 0.38$		$p = 0.09$		$p = 0.04$	

The *p*-values shown are obtained from a two-tailed paired *t*-test (without correction for multiple comparisons).

Table 2

Inter-subject variations (defined as the ratio between standard deviation and group mean) before and after normalization with global CVR ($N = 20$)

Region Of Interest	Va (Unitless)	Vr (Unitless)	(Va – Vr)/Va (%)
Ins	0.22	0.15	32.8
Fro	0.26	0.18	31.4
Cen	0.26	0.14	46.5
Lim	0.26	0.15	43.4
ScG	0.27	0.21	19.9
Tem	0.18	0.06	65.3
Par	0.22	0.09	57.7
Occ	0.21	0.12	42.4
Cer	0.25	0.10	57.5

Regions of interest are insular cortex (Ins), frontal cortex (Fro), central sulcus (Cen), limbic cortex (Lim), subcortical gray nuclei (ScG), temporal cortex (Tem), parietal cortex (Par), occipital cortex (Occ), and cerebellum (Cer).

Va, variations in absolute CVR; Vr, variations in relative CVR.