

## Detection and scaling of task-induced fMRI-BOLD response using resting state fluctuations

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This study evaluated a calibration technique for scaling the fMRI-BOLD response during a simple motor task. A novel scaling parameter, resting state physiological fluctuation amplitude (RSFA), was tested using previously established scaling factors such as breath hold or 5% CO<sub>2</sub>. RSFA was hypothesized to contain vascular reactivity information present in the resting state fMRI signal. Subjects were scanned under various stimulus conditions: (a) rest while breathing room air, (b) bilateral fingertapping, (c) breath holding and (d) moderate hypercapnia (breathing 5% CO<sub>2</sub> + air). In all subjects who breathed 5% CO<sub>2</sub>, RSFA correlated highly with the BOLD response amplitude during 5% CO<sub>2</sub> inhalation. Also, RSFA correlated highly with the amplitude of the BOLD response elicited by breath hold. RSFA was therefore used as a hemodynamic scaling factor to calibrate both the amplitude and spatial extent of the fMRI-BOLD response during the motor task (fingertapping). Results revealed that amplitude scaling using RSFA was similar to that using breath hold or 5% CO<sub>2</sub>, where the spatial extent of activation diminished by 20–37% over all subjects. Spatial extent of activation changed significantly after scaling and only 30–40% of the activated area overlapped with the unscaled activation. RSFA-scaled task-induced fMRI-BOLD response in both amplitude and spatial extent was comparable to that obtained using breath hold or 5% CO<sub>2</sub>. We conclude that RSFA may be used to hemodynamically scale the fMRI-BOLD response and does not require the use of a hypercapnic challenge (which may not be purely non-neural), which can be difficult to implement in special populations.

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### Introduction

One of the main challenges facing functional magnetic resonance imaging (fMRI) is to reliably detect and quantify task-activated signal arising from capillaries which are closest and

physiologically coupled with neural activity while minimizing signals from large arteries and draining veins. This challenge occurs because of the strong weighting of the MR-T2\* signal by blood volume in each voxel (Boxerman et al., 1995; Menon, 2002). Thus, fMRI-BOLD signal change can be a result of an increase in neural activity convolved with intrinsic vascular sensitivity (the ability of blood vessels to constrict or dilate). This leads to variations in the activation-induced BOLD responses across subjects depending on their vascular sensitivity. Variation may become more pronounced in older subject populations due to compromised vascular sensitivity as a result of aging or disease (Riecker et al., 2003). Large variation in hemodynamic properties exists between subjects and even between brain regions within subjects and this natural variability can reduce confidence in regional or group activation differences during task performance (Huettel and McCarthy, 2001; D'Esposito et al., 1999). To overcome some of these limitations in fMRI-BOLD response, a number of research groups have attempted to characterize regional hemodynamic differences by inducing physiological perturbations that stimulate hemodynamic activity without significantly altering neural activity (Kastrup et al., 1999; Li et al., 1999; Kannurpatti et al., 2002). One such physiological perturbation is to induce mild to moderate hypercapnic response via intermittent breath holding or inspiration of a CO<sub>2</sub>/air mixture. These hypercapnic methods permit scaling of task-induced signal changes to account for regional vascular differences (Bandettini and Wong, 1997; Davis et al., 1998; Cohen et al., 2004). Recently, breath hold procedures were used as a hypercapnic task to minimize variability in fMRI-BOLD response in young or aging populations (Thomason et al., 2007; Handwerker et al., 2006). These methods however require subjects to hold their breath for an extended time, which may be difficult for patients, children or elderly to perform (Thomason et al., 2005). A further problem with hypercapnia methods is that they can possibly bias the outcome of hemodynamic scaling. This depends on the evoked neural activation confounds inherent within the hypercapnia task such as breathing CO<sub>2</sub> or breath hold (Corfield et al., 1995; Banzett et al., 2000; Brannan et al., 2001; Liotti et al., 2001; Parsons et al., 2001; Evans et al., 2002; Cohen et al., 2004). Thus, scaling of the fMRI-BOLD response to the vascular sensitivity information contained in the task response

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signal, or during resting conditions can avoid scaling bias and subject compliance issues related to the hypercapnic task.

In the present study, we have scaled the fMRI-BOLD response to a motor task in a group of human subjects using RSFA and compared it with prior scaling variables such as breath hold or breathing CO<sub>2</sub>. We have tested the hypothesis that RSFA reflects the underlying cerebral vascular reactivity to CO<sub>2</sub> variations or hypercapnia in the arterial system.

## Methods

### Subjects

Eight healthy volunteers (3 females, 5 males) between the ages of 23–67 years (mean age=43 years) with no history of head trauma, neurological disease or hearing disability participated in the study. All protocols in this study were reviewed by the Institutional Review Board at the University of Medicine and Dentistry of New Jersey. Written consent was obtained from all volunteers after the nature and possible consequences of the study were explained to them.

### fMRI parameters and tasks

All images were obtained on a Siemens Allegra, 3-T MR scanner. The imaging system was equipped with a fixed asymmetric head gradient coil and a shielded end cap quadrature transmit/receive birdcage radio-frequency coil. Volunteers were positioned supine on the gantry with their head in a midline location in the coil. To reduce motion artifacts, foam padding was placed between the forehead and the RF-coil. In each volunteer, echo-planar images were obtained across the motor cortex in the axial plane using a 64×64 matrix, TR/TE=1 s/27.2 ms, FOV=20 cm, 6 slices, slice thickness=7 mm and a bandwidth of 125 kHz. A flip angle of 80° was used to minimize flow weighting. The imaging procedure for each volunteer was as follows: sagittal localizer images were first obtained with a conventional gradient echo sequence and the mid-sagittal image was used to select six axial slices covering the motor cortex for functional imaging.

Hypercapnia or fingertapping tasks were delivered as a block design paradigm. For the fingertapping paradigm, subjects were at rest for the first 25 s after start of MR acquisition, followed by three alternating 25-s periods of bilateral fingertapping and 50-s periods of rest. During the fingertapping task, subjects were instructed to successively touch each finger with the thumb of the respective hand in a self-paced manner. Fingertapping rate varied from 0.5 and 2 taps per second across subjects. During the hypercapnia task (breath hold), subjects breathed normally for the first 25 s followed by three alternating 25-s periods of breath hold and 50-s periods of normal breathing. During the hypercapnia task (breathing 5% CO<sub>2</sub>), subjects breathed normal room air for the first 25 s followed by three alternating 25-s periods of breathing air+5% CO<sub>2</sub> and 50-s periods of breathing room air. For each experimental condition, namely resting, finger tapping, breath hold or breathing 5% CO<sub>2</sub>, 250 echo planar images were obtained for a total scan time of 250 s. The subjects were instructed verbally through a microphone and speaker system at the time of the onset of each of the separate tasks. Subjects rehearsed all tasks (except breathing 5% CO<sub>2</sub>) outside the scanner, before the experimental session and received instructions to maintain constant attention

during the experiments. Four subjects declined to receive 5% CO<sub>2</sub> but performed the breath hold task.

### Data analysis

fMRI data for all experimental runs were preprocessed using AFNI (Cox, 1996). Reconstructed echo planar images were corrected for motion using a rigid-body volume registration. Echo planar images of breath hold and CO<sub>2</sub> scans were registered to fingertapping scans using 3dvolreg in AFNI (Cox, 1996). Data were examined for temporal drifts in the BOLD signal time series by comparing the mean intensities of the echo-planar image numbers 5–10 with the image numbers 245–250. BOLD signal drift within the temporal duration of experimental scan was less than 0.5% of the mean baseline BOLD signal intensity. Voxel-wise correlation of signal intensity time course with a boxcar reference function was used to determine activation using a threshold of 0.4 for the correlation coefficient (Bonferroni corrected  $p$ -value  $p < 10^{-6}$ ) (Bandettini et al., 1993). The boxcar function was appropriately shifted to take into account the hemodynamic delay. For each voxel, the percent change in the BOLD signal intensity was calculated as the standard deviation (SD) of the BOLD signal time series for the respective experimental condition.

### Hemodynamic scaling of task-induced response

In accordance with the model established by Davis et al. (1998) and Hoge et al. (1999), the relationship between CBF, CMRO<sub>2</sub> and BOLD signal can be determined as:

$$B_{act} = M[1 - (CMRO_{2act}/CMRO_{2rest})^\beta \cdot (CBF_{act}/CBF_{rest})^{\alpha-\beta}] \quad (1)$$

where  $B_{act} = \Delta BOLD/BOLD_{rest}$  is the BOLD signal change in response to a task,  $M$  is a constant dependent on the vasomotor properties,  $\alpha$  and  $\beta$  are constants.  $\alpha = 0.38$  for normocapnic conditions (Grubb et al., 1974), while  $\beta$  can vary between 1 and 2 depending on the susceptibility and contribution from intra- and extravascular compartments which can vary with the field strength. A value of  $\beta = 1$  can be approximated for a field strength of 3 T.

It was assumed that no change in CMRO<sub>2</sub> occurred during the breath hold task. Thus, during breath hold, 5% CO<sub>2</sub> breathing or resting state scan conditions where no evoked neural activity occurred, Eq. (1) can be simplified as:

$$B_{BH} = M[1 - (CBF_{BH}/CBF_{rest})^{\alpha-\beta}] \quad (2)$$

where  $B_{BH} = \Delta BOLD/BOLD_{rest}$  was the BOLD signal change in response to breath hold, breathing CO<sub>2</sub> or during resting conditions. The multiplicative constant  $M$  was the same for both task activated and breath hold conditions when considered from a similar spatial location in the brain. Thus, division of Eq. (1) by (2) led to a measurable indicator of cerebral metabolic activity, which can be defined as the scaled fMRI-BOLD response. As  $F_{act}/F_{BH} \approx 1$  for a strong sensory/motor task (Hoge et al., 1999; Kastrup et al., 2002) and change in CBF was between 2 and 5 times CMRO<sub>2</sub> (Fox and Raichle, 1984; Hoge et al., 1999), the scaled fMRI-BOLD signal would be proportional to cerebral metabolic activity with minimized variations from vascular sensitivity defined by  $M$  in Eqs. (1) and (2).

A scaling index defined by the value according to Eq. (2) was obtained for every voxel in the brain. Scaling indices  $B_{BH}$ ,  $B_{CO_2}$  and  $B_{RSFA}$  were obtained from the temporal SD in BOLD signal during breath hold, CO<sub>2</sub> breathing and resting state BOLD signal time

series respectively. The resting state data were low pass (0.1 Hz cutoff) and high pass filtered (0.1 Hz cutoff) and the SD of these filtered data was compared with the unfiltered data. SD of the unfiltered time series correlated highly with the SD of low and high pass filtered time series ( $R^2=0.93$  and  $R^2=0.91$ , respectively). Further, the SD value in each voxel decreased by less than 15% with low and high pass filtering when compared to the voxel SD value without any filtering. Hence, unfiltered signals were considered to determine the scaling factor. The percent change in the BOLD signal during the neural activation task was calculated as the SD of the BOLD signal time series during the fingertapping scan. The percent change in the BOLD signal over voxels that were significantly active after the voxel-wise cross-correlation analysis formed the unscaled BOLD activation map. Amplitude scaling was accomplished by simply dividing the percent BOLD signal change during the fingertapping task by the appropriate scaling index on a voxel-wise basis.

#### Hemodynamic scaling on the spatial extent of activation

To compare activation volume before and after hemodynamic scaling, activation maps were determined using the top 1% of the BOLD signal change values from the respective null distribution as the threshold. In a separate analysis, spatial overlap of activated regions before and after hemodynamic scaling was determined. For this analysis, the top 1% of the BOLD signal change values from the respective activation distributions were considered. Regions of overlap in activation were obtained by converting highest activated regions for each experimental condition into binary maps. Overlapped regions were detected on a voxel-wise basis using a logical and operation on the two binary masks. Percent overlap was calculated as the ratio of active voxels in the resulting mask after an AND operation to the active voxels in the resulting binary mask after an OR operation.

#### Results

BOLD signal change during breath hold or 5% CO<sub>2</sub> was larger in the gray matter compared to the white matter and was pronounced over multiple anatomical regions in the brain. Assuming that the intensity increase in the T2\*-weighted images during breath hold or 5% CO<sub>2</sub> are a hypercapnic cerebral vasodilatory response without significant neural activation, the spatio-temporal extent of the BOLD signal during hypercapnia was measured over all subjects. BOLD signal response during breath hold or 5% CO<sub>2</sub> correlated highly with RSFA on a voxel-wise basis. As depicted in Figs. 1a and b from a typical subject, a high correlation was observed between BOLD signal change during breath hold or 5% CO<sub>2</sub> with RSFA from the corresponding region within the brain. Furthermore, the highest correlation was observed between breath hold and 5% CO<sub>2</sub> from corresponding regions within the brain (Fig. 1c) and over all subjects (Table 1). The high correlation between breath hold or 5% CO<sub>2</sub> with RSFA was reproducible over all subjects (Table 1). Blood oxygen saturation monitored by a pulse oximeter did not change significantly during breath hold or 5% CO<sub>2</sub> in the monitored subjects ( $n=4$ ). The average resting blood oxygen saturation in the monitored subjects was 97%.

#### Hemodynamic scaling of the BOLD signal amplitude

Finger tapping-induced fMRI-BOLD response was detected in the motor cortex across all subjects. Activation maps were detected

after cross-correlating the reference waveform with the measured BOLD signal time series. Fig. 2a shows typical activation (percent change in BOLD signal amplitude) in response to a bilateral fingertapping task from four contiguous axial slices covering the motor cortex. Several voxels with large BOLD signal change can be distinguished from the activated region of interest (ROI) prior to hemodynamic scaling. Average increase in BOLD signal intensity in the activated region across all subjects was  $3.38 \pm 1.77\%$ . The activated ROI prior to hemodynamic scaling was used as a mask for subsequent amplitude scaling analysis using the different scaling variables.

BOLD responses to fingertapping task were subsequently divided by RSFA, breath hold or 5% CO<sub>2</sub> on a voxel-wise basis to obtain a scaled fMRI-BOLD measure indicative of cerebral metabolism. Established hypercapnic scaling parameters such as BOLD signal response during breath hold or breathing 5% CO<sub>2</sub>

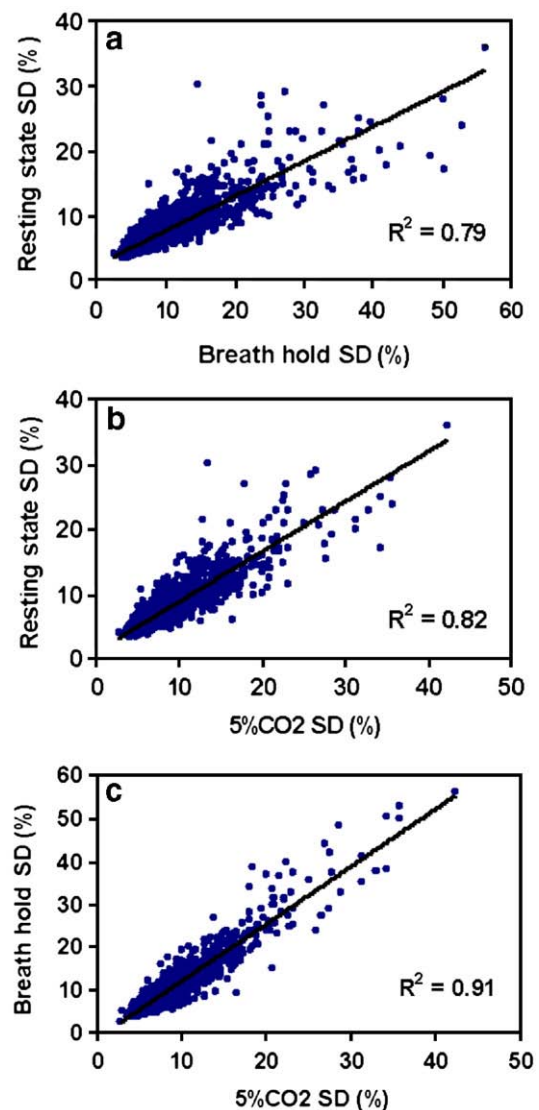


Fig. 1. Correlation between (a) RSFA vs. breath hold response, (b) RSFA vs. 5% CO<sub>2</sub> response, and (c) breath hold vs. 5% CO<sub>2</sub> response in a typical subject. The peak-to-peak amplitude (standard deviation—SD) of the BOLD signal in each condition was determined on a voxel-wise basis from all slices covering the motor cortex to determine the correlations.

Table 1  
Correlation between RSFA vs. breath hold, RSFA vs. 5% CO<sub>2</sub> and breath hold vs. 5% CO<sub>2</sub> over all subjects (four subjects breathed 5% CO<sub>2</sub>)

Subject	Correlation ( $R^2$ ) RSFA vs. breath hold	Correlation ( $R^2$ ) RSFA vs. 5% CO <sub>2</sub>	Correlation ( $R^2$ ) breath hold vs. 5% CO <sub>2</sub>
1	0.78	0.78	0.87
2	0.89	0.92	0.96
3	0.88	0.89	0.92
4	0.74	0.69	0.88
5	0.81	–	–
6	0.88	–	–
7	0.87	–	–
8	0.79	–	–
Group average	0.83±0.06	0.82±0.11	0.91±0.04.

were used to validate the novel scaling parameter, RSFA. The SD of the BOLD signal time series during the fingertapping task-induced responses was divided by the SD of the vasoactive stimuli-

induced response namely breath hold, 5% CO<sub>2</sub> or RSFA. The activated ROI determined before any hemodynamic scaling (Fig. 2a) was considered as the mask. As observed in Figs. 2b–d, hemodynamic scaling using RSFA, breath hold or 5% CO<sub>2</sub> reduced the fingertapping-induced BOLD response. Histograms of the unscaled and scaled distributions indicated that percent change within the activated ROI was more consistent after hemodynamic scaling. Fig. 3 shows the unscaled and scaled distributions from the activated region of interest before hemodynamic scaling from a typical subject. A similar trend was observed across all subjects (Table 2). Hemodynamic scaling using RSFA, breath hold or 5% CO<sub>2</sub> significantly reduced group average of the BOLD signal change in response to fingertapping by 60% and group SD by 85%. This reduction was comparable across all three scaling variables though a significantly larger extent of scaling was observed for breath hold when compared to RSFA or 5% CO<sub>2</sub> (Table 2;  $p < 0.007$ , paired  $t$ -test). The overall extent of amplitude scaling obtained using RSFA was comparable to that obtained with breath hold or 5% CO<sub>2</sub> (Table 3). As the ROI defined by voxel-based

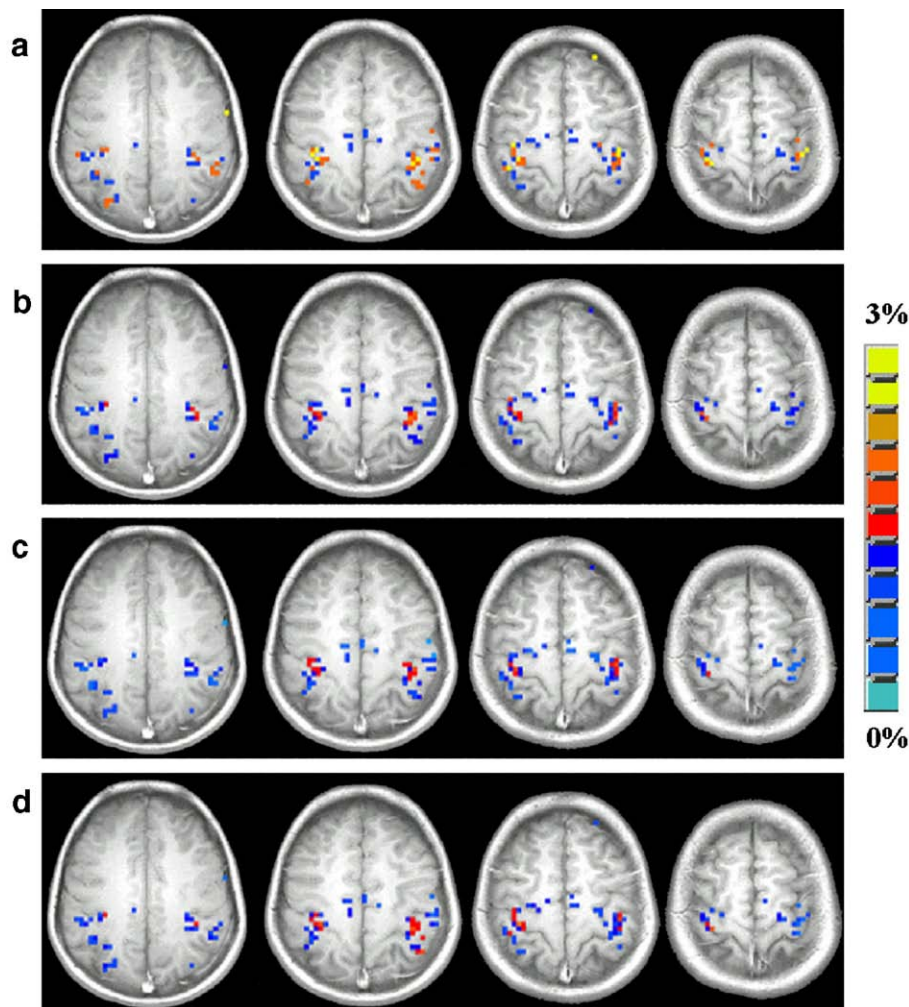


Fig. 2. Activation maps of the fingertapping experiment superimposed on the anatomical image in a typical subject. (a) Prior to hemodynamic scaling, (b) scaled using RSFA, (c) scaled using breath hold and (d) scaled using 5% CO<sub>2</sub>. Activation maps were generated as described in methods. Activation was determined by cross-correlating the task-induced BOLD response time series on a voxel-wise basis with a reference function similar to the task. Voxels with a correlation value of 0.4 or greater (adjusted  $p$ -value,  $p < 10^{-6}$ ) were considered activated. The colors in the activation map determine the percent BOLD signal change in response to the fingertapping task.

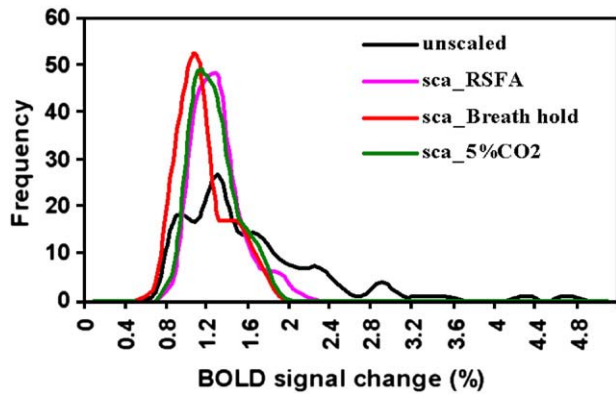


Fig. 3. Distribution of the unscaled and scaled BOLD response to a fingertapping task from a typical subject. The activated region was determined from the unscaled BOLD signal response as described in Fig. 2. The unscaled activated region was used as a mask to obtain the scaled values from the same voxel locations after the BOLD response to the finger tapping task was scaled using various scaling parameters namely RSFA, breath hold or 5% CO<sub>2</sub>. The scaled responses are distinctly narrow when compared to the unscaled distribution. The distributions after scaling with RSFA, breath hold or 5% CO<sub>2</sub> were very similar. A similar trend was observed over all subjects and is shown in Table 2.

cross-correlation threshold was the same for different conditions, rescaling helped in comparing amplitude responses between conditions for a given group of voxels. Each distribution curve in Fig. 3 shows the effects between voxels for a given condition.

#### Hemodynamic scaling of the spatial extent

Distribution over all voxels of the percent change in BOLD signal, in response to fingertapping, was obtained before and after hemodynamic scaling. Activation maps of the percent change in BOLD signal amplitude were obtained using the top 1% values of the BOLD signal change from the respective null distribution as the threshold. A comparison of spatial extent of finger tapping-induced BOLD response, in a typical subject, before and after scaling with the various scaling factors is shown in Fig. 4. A 75% decrease in activation volume after hemodynamic scaling with breath hold or 5% CO<sub>2</sub> was observed over all subjects when

Table 2  
BOLD signal amplitude in response to a fingertapping task within the activated ROI before and after scaling with different scaling parameters

Subject	Before scaling	Scaling with RSFA	Scaling with breath hold	Scaling with 5% CO <sub>2</sub>
1	4.15±5.45	1.04±0.24	1.27±0.35	1.35±0.34
2	2.77±3.30	1.36±0.27	0.97±0.26	1.22±0.27
3	2.09±4.22	1.32±0.24	1.17±0.27	1.28±0.25
4	7.28±11.66	1.35±0.31	0.81±0.26	0.97±0.39
5	2.74±3.93	1.51±0.41	1.12±0.30	–
6	3.88±6.49	1.36±0.33	0.92±0.27	–
7	1.79±1.18	1.34±0.30	1.14±0.28	–
8	2.34±1.89	1.65±0.47	1.04±0.22	–
Group average	3.38±1.17	1.37±0.17	1.06±0.15*	1.20±0.17**

Values are the mean±SD.

\* Significantly different with respect to RSFA,  $p < 0.007$ , paired  $t$ -test.

\*\* Significance with respect to RSFA,  $p < 0.07$ ,  $t$ -test equal variance.

Table 3

Variability (ratio of SD/mean) of the fingertapping response within the activated ROI before and after scaling with different scaling parameters

Subject	Before scaling	Scaling with RSFA	Scaling with breath hold	Scaling with 5% CO <sub>2</sub>
1	1.31	0.23	0.28	0.25
2	1.19	0.19	0.27	0.22
3	2.01	0.18	0.23	0.20
4	1.60	0.23	0.32	0.40
5	1.43	0.27	0.26	–
6	1.67	0.24	0.29	–
7	0.66	0.22	0.25	–
8	0.81	0.28	0.21	–
Group average	1.33±0.45	0.23±0.03	0.26±0.03	0.27±0.09

compared to activation volume prior to scaling (Table 4). However, no significant difference was observed in activation volume after scaling with RSFA.

In a separate analysis, spatial overlap of a fixed population of voxels (determined using the top 1% values of the BOLD signal change from the respective activation distribution as the threshold) was compared over different experimental conditions. Spatial overlap of the unscaled with scaled activation, on a subject wise basis, was performed using the top 1% of the responding pixels. A mean overlap of 29–34% was observed using RSFA, breath hold or 5% CO<sub>2</sub> (Table 5). This indicated a significant alteration in spatial location of the highly responding voxels during the neural task after hemodynamic scaling when compared to the unscaled condition.

#### Discussion

fMRI-BOLD activation is the T2\*-weighted signal intensity, which is dependent on the difference between oxyhemoglobin and deoxyhemoglobin. Thus, a change in T2\* signal during a task is indicative of underlying neuronal activation (Logothetis et al., 2001). However, fMRI-T2\* signal change representative of neuronal activity is influenced by the sensitivity of the vascular system that couples neural activity with blood flow. This makes the signal intensity change in each voxel dependent on variations in the intrinsic vascular sensitivity (e.g., ability of blood vessels to constrict or dilate). Further, different brain regions have varying vascular anatomy and caliber, which leads to spatial variation of BOLD signal depending on the underlying cerebrovascular or cerebrometabolic activity (e.g., high BOLD signal change in large vessels).

Based upon the assumption that oxygen consumption remains stable during mild hypercapnia (Yang and Krasney, 1995), hypercapnia has been used to improve functional activation by minimizing contribution from cerebrovascular components of BOLD signal change (Bandettini and Wong, 1997; Davis et al., 1998; Cohen et al., 2004). Hypercapnic stimuli in humans have been delivered using either 5% CO<sub>2</sub> from a gas source connected to a face-mask worn by the subject or using a breath hold task (Bandettini and Wong, 1997; Kastrup et al., 1999; Li et al., 1999; Kannurpatti et al., 2002). As insufficient subject cooperation regarding task performance in special populations such as the elderly and children is an important concern in fMRI studies (Thomason et al., 2005). Breath hold has the advantage of inducing a hypercapnic stimulus without the subject requiring a mask. Several studies have reported excellent patient tolerance with

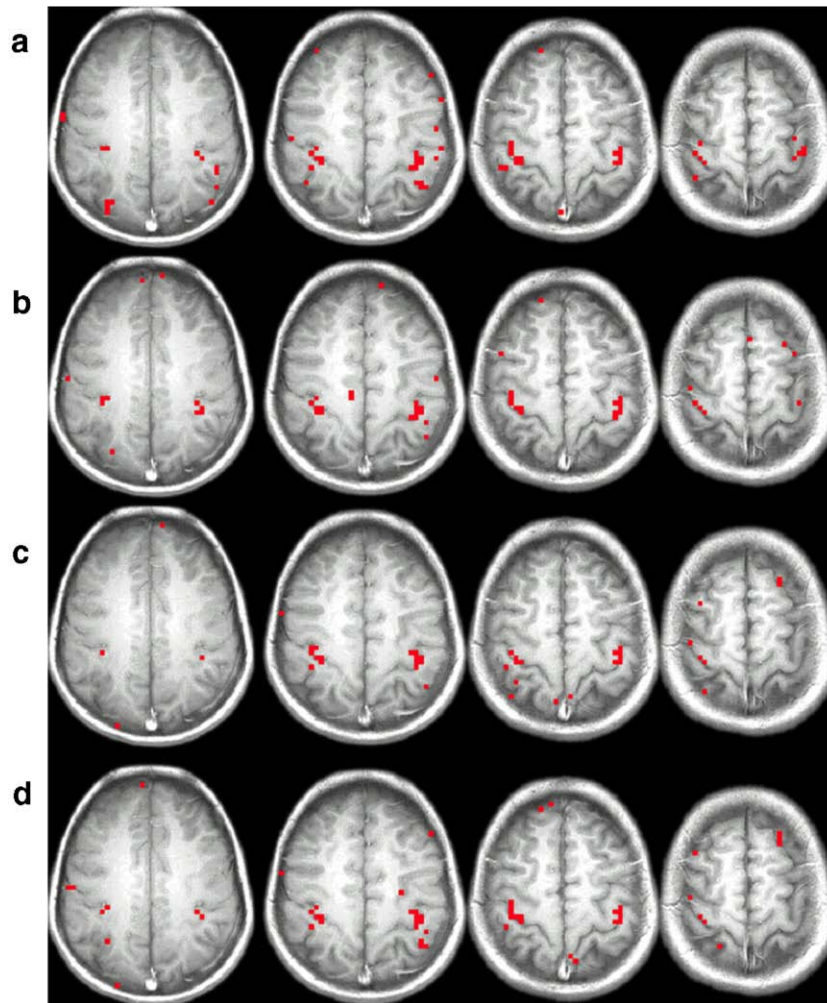


Fig. 4. Spatial extent of activation before and after hemodynamic scaling in three contiguous axial slices covering the motor cortex. Activation was determined using a 99th percentile threshold from the null distribution of the BOLD signal amplitude change ( $p < 0.01$ ). (a) Before and (b–d) after scaling with different variables namely (b) RSFA, (c) breath hold and (d) 5%  $\text{CO}_2$ . Decreased activation volume can be distinguished after scaling using RSFA, breath hold or 5%  $\text{CO}_2$ . Data shown are from a typical subject.

breath hold and even less problems in patients for whom cooperation regarding task performance is an issue (Stoll et al., 1996; Beisteiner et al., 2000). Thus breath hold has become a popular hypercapnic stimulus and has been recently used as a scaling factor to remove vascular sensitivity variations in subjects performing a cognitive or a visual task (Thomason et al., 2007; Handwerker et al., 2006).

A separate but critical concern with hypercapnic scaling of the BOLD signal is the potential to cause sensory stimulation and therefore brain activation due to air hunger (Corfield et al., 1995; Banzett et al., 2000; Brannan et al., 2001; Liotti et al., 2001; Parsons et al., 2001; Evans et al., 2002). Spontaneous breathing of  $\text{CO}_2$  gas mixtures, when distinguishable from breathing normal air, tends to be associated with sensations of an urge to breathe, rapid breathing and increased respiratory work (Simon et al., 1989; Moosavi et al., 2003), especially at high end-tidal  $\text{CO}_2$ . Thus, hemodynamic scaling with such a stimulus may not reflect scaled task signal change, which corresponds to neural metabolism (Cohen et al., 2004). Thus calibration of the BOLD signal warrants a scaling parameter, free of evoked neural activity.

Subtle change in a subject's breathing rate or depth, which occurs naturally during rest at low frequencies ( $< 0.1$  Hz), has been shown to be significantly correlated with fMRI signal change throughout gray matter and near large vessels (Birn et al., 2006). This is related to fluctuations in arterial  $\text{CO}_2$ , which induce a significant low frequency variation in the BOLD signal (Wise et al., 2004). We hypothesized that RSFA is an indicator of arterial  $\text{CO}_2$  variation and correlated BOLD signal change during a breath hold or breathing 5%  $\text{CO}_2$  condition with RSFA. A high correlation was observed between breath hold or 5%  $\text{CO}_2$  with RSFA in all subjects (Table 1). The slope of the scatter plot of breath hold vs. 5%  $\text{CO}_2$  was approximately 1, which indicated that the two hypercapnic stimuli (breath hold or 5%  $\text{CO}_2$ ) were equivalent, whereas the slope of RSFA vs. breath hold or RSFA vs. 5%  $\text{CO}_2$  was less than 1. This indicated that the variation in the baseline fMRI-BOLD signal during resting conditions, presumably due to change in arterial  $\text{CO}_2$  was less than the arterial  $\text{CO}_2$  changes that may have occurred during the breath hold or 5%  $\text{CO}_2$  task. Based on the high correlation between breath hold or 5%  $\text{CO}_2$  with RSFA, we evaluated the robustness of RSFA as a hemodynamic

Table 4  
Spatial extent of activation (activation volume in cm<sup>3</sup>) in response to the fingertapping task over all subjects before and after hemodynamic scaling with various parameters

Subject	Before scaling	Scaling with RSFA	Scaling with breath hold	Scaling with 5% CO <sub>2</sub>
1	20.8	4.0	2.5	3.0
2	7.2	3.5	1.8	2.6
3	76.2	49.3	3.1	18.1
4	17.9	11.5	1.5	3.7
5	55.4	87.3	29.3	–
6	17.2	22.5	1.7	–
7	2.9	21.8	5.2	–
8	31.3	47.9	5.3	–
Group average	28.6±25.1	30.9±29.4	6.3±9.4*	6.9±7.5**

\* Significantly different with respect to RSFA,  $p < 0.009$ , paired  $t$ -test.

\*\* Significantly different with respect to RSFA,  $p < 0.02$ ,  $t$ -test unequal variance.

scaling parameter. As breath hold or 5% CO<sub>2</sub> response amplitude has previously been used to minimize the cerebrovascular component in task activated data (Bandettini et al., 1997; Thomason et al., 2007; Handwerker et al., 2006) we tested the novel hemodynamic scaling variable RSFA with established hemodynamic scaling variables, breath hold and 5% CO<sub>2</sub>.

In all subjects, mean and SD of the hemodynamically scaled response to a fingertapping task was reduced to a comparable extent with either RSFA, breath hold or 5% CO<sub>2</sub> when compared to the unscaled distribution (Table 2 and Fig. 3). Variability in fingertapping response was reduced by almost 80% after scaling with RSFA, breath hold or 5% CO<sub>2</sub>. Theoretically, the extent of reduction in group variance after scaling is defined by the relation  $\sigma_{\text{sca}} = [1 - r^2]^{1/2} \sigma_{\text{act}}$ , where  $r^2$  is the correlation between hypercapnic task and the neuronal task-induced signal change for the group while  $\sigma_{\text{sca}}$  and  $\sigma_{\text{act}}$  are the SDs of the neuronal task-induced activations after scaling and prior to scaling, respectively (Thomason et al., 2007). With mean correlation between task (finger tapping) and hypercapnic conditions (RSFA, breath hold or 5% CO<sub>2</sub>) exceeding 0.92 in the group of subjects (data not shown), a reduction in the group SD of approximately 70% may be expected. As indicated by our results, approximately an 85% reduction in the group SD was observed after scaling with RSFA, breath hold or 5% CO<sub>2</sub> (Table 2). The extent of reduction in variability after scaling the motor task response in this study was significantly larger than the 25% reduction in inter-subject BOLD variability observed in a working memory task (Thomason et al., 2007). Also a 3.4% signal change that occurred with a broad distribution of activated voxels across the sensorimotor cortex during fingertapping reduced to 1.2% after hemodynamic scaling was reflected by a greater correlation between hypercapnic and finger tapping response. As the BOLD signal amplitude change in response to the motor task was relatively larger when compared to a cognitive task (Thomason et al., 2007; Handwerker et al., 2006), the vascular component contributing to the signal change would be large for higher amplitude BOLD responses in the brain.

Activation maps of percent change in BOLD signal were obtained using the top 1% of BOLD signal response values from the respective null distribution as the threshold. Activation volume after hemodynamic scaling was not significantly different from the ones prior to scaling except with breath hold and 5% CO<sub>2</sub>, where

activation volume decreased by 75% after hemodynamic scaling with breath hold or 5% CO<sub>2</sub> (Table 4). As significant statistical power is lost while considering only the amplitude to determine a statistical threshold in comparison to cross-correlation analysis, which contains temporal structure of signal change, activation volume comparisons with hemodynamic scaling using the different scaling variables was not corrected for multiple comparisons. Mean spatial overlap of scaled activation was 29–34% over all three variables, which indicated that vascular variability significantly altered spatial extent of activation. Thus spatial pattern due to actual neural metabolism may be distinct from the unscaled BOLD response. As a fixed number of 65 highly activated pixels (the top 1% of a total of 6500) were considered over different experimental conditions to determine spatial overlaps, activated regions in a sense simply reflected the pattern of largest BOLD signal percent change voxels.

## Conclusion

Breath hold, breathing 5% CO<sub>2</sub> or RSFA used as hemodynamic scaling parameters significantly reduced variation in neural task-induced fMRI-BOLD response amplitude within subjects and across subjects. RSFA correlated well with BOLD signal variation induced by a breath hold task or breathing 5% CO<sub>2</sub>. This suggests that information contained within the resting state baseline BOLD signal in the form of fluctuations depends on systemic CO<sub>2</sub> changes. Hemodynamic amplitude scaling accomplished using RSFA was comparable to that obtained using breath hold or 5% CO<sub>2</sub>. Reduced spatial overlap of activation after hemodynamic scaling compared prior to scaling suggests that the true BOLD signal change due to underlying neural metabolism is spatially distinct. RSFA as a hemodynamic scaling factor would eliminate the necessity to undergo an additional hypercapnic task, which is difficult to perform in special populations such as patients, children and the elderly.

Despite motion, scanner instabilities and CSF pulsations that may distort the correlation of SD with cerebrovascular reactivity to CO<sub>2</sub>, the most significant benefit with RSFA would be the scaling of fMRI-BOLD response that exactly reflect evoked neural activity without neural confounds associated with breathing CO<sub>2</sub> or breath hold. Scaling with RSFA will also eliminate the need to arbitrarily select hypercapnic stimulus levels (e.g., 5% CO<sub>2</sub> or 20-s breath hold) as it is not known what level of hypercapnia can be suitable to obtain the ideal hemodynamic scaling factor.

Table 5  
Overlap in the spatial extent of activation during the fingertapping task over all subjects before and after hemodynamic scaling

Subject	Percent overlap in the spatial extent of activation		
	Unscaled vs. scaled_RSFA	Unscaled vs. scaled_brhld	Unscaled vs. scaled_5% CO <sub>2</sub>
1	36	39	38
2	26	20	29
3	42	36	41
4	37	28	26
5	32	17	–
6	28	46	–
7	23	26	–
8	19	18	–
Group average	30.4±7.7	28.8±10.6	33.5±7.1

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