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Hemodynamic Scaling of fMRI-BOLD signal: Validation of low frequency spectral amplitude as a Scalability Factor

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Abstract

fMRI-BOLD signal representing neural activity may be optimized by discriminating MR signal components related to neural activity and those related to intrinsic properties of the cortical vasculature. The objective of this study was to diminish the hemodynamic change independent of neural activity to obtain a scaled fMRI-BOLD response using two factors namely the low frequency spectral amplitude (LFSA) and breath hold amplitude (BHA). Ten subjects (22-38 years of age) were scanned during various task conditions; (a) rest while breathing room air; (b) bilateral finger-tapping breathing room air; (c) rest during a partial inspirational breath hold; and (d) rest during moderate hypercapnia (breathing 5% CO₂, 20% O₂, and 75% N₂). In all subjects who breathed 5% CO₂, regions with significant BOLD response during breath hold correlated significantly with the percent signal increase during 5% CO₂ inhalation. Finger tapping-induced responses in the motor cortex were diminished to a similar extent after scaling using either the LFSA or the BHA. Inter and intra-subject variation in the amplitude of the BOLD signal response reduced after hemodynamic scaling using LFSA or BHA. The results validate the hemodynamic amplitude scaling using the LFSA with earlier established BHA. LFSA free from motor task contamination can be used to calibrate the fMRI-BOLD response in lieu of BHA or hypercapnia to minimize intra and inter subject variation arising from vascular anatomy and vasodilative capacity.

Keywords

fMRI; breath hold; BOLD; CBF; scaling; hypercapnia; low frequency; spectral amplitude

Introduction

Functional magnetic resonance imaging (fMRI) permits measurement of local changes in cerebral blood volume [1], flow [2], and oxygenation [3,4] in cortical vasculature, secondary to task-related neural activity. To infer neural activity, most fMRI studies use T2*-weighted pulse sequences that are sensitive to blood oxygenation level dependent (BOLD) signal change because T2* has a high contrast-to-noise ratio and does not need the administration of contrast agents. While the mechanisms that govern the relationship between neural activity and BOLD response are rudimentary, evidence suggests that neural activity leads to vasodilation and

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increased blood flow [2], possibly by glutamatergic and GABAergic neurovascular signal propagation [5]. The resulting net increase in the difference between oxyhemoglobin and deoxyhemoglobin [3,4] gives rise to greater MR signal intensity in activated regions which reflects the input and intracortical processing of the given brain region [6].

Vascular properties may affect the validity of fMRI analysis

The validity of fMRI analysis methods depends upon the extent to which MRI signal changes faithfully represent underlying task-related neuronal activity. Under favorable conditions, the task-induced signal changes by about 5-10% over the mean signal intensity obtained during baseline portions of the time-course. Across the time-course, baseline signal intensity can vary by as much as 1%. fMRI-BOLD signal changes across regions result, not only from neural activity related to stimulus presentation or task performance, but also from vascular factors including “vascular compliance” (i.e., the tendency of arterial tissue to dilate in response to neural activity) and vascular density i.e., the distribution of capillary-bed density over cortex [7,8]. Thus, valid detection and quantification of fMRI signal must take into account, not only the task-activated “signal” arising from capillary beds, but also signal variation due to several hemodynamic sources including relative vascular density, vascular sensitivity and proximity to large arteries and draining veins as MR-T2* signal is strongly weighted by the blood volume in each voxel.

Vascular density and vascular compliance varies between brain regions

Vascular density is known to vary between brain regions [7,9,10]. In one study for instance, Harrison et al., [7] observed considerable variation in capillary-bed density across temporal cortex with some regions densely innervated while others fairly sparse. Proximity of dense vasculature to regions of neural activity would be expected to compromise spatial resolution of fMRI signal. Further, interactions between capillary bed distributions and their orientation relative to the magnetic field (e.g., whether the capillaries are parallel or perpendicular to the magnetic field), could affect signal detection sensitivity. This is because the volume of protons excited by any given RF pulse will vary depending on the density, distribution, and orientation of the vascular elements including vessel caliber [11].

Another important factor that may affect fMRI accuracy is vascular compliance. Vascular compliance may be defined as the tendency of arteriovenous tissue to dilate in response to various second messengers, O₂ depletion and CO₂ accumulation that trigger neural-related hemodynamic response [5,12-14]. Variability in vascular compliance may lead to variation in blood flow velocity. In one study for instance, blood flow velocity was measured by video microscopy using an injected fluorescent compound that labeled red blood cells [15]. Using video microscopy and frame-by-frame analysis, the authors observed that, during 5% CO₂ ventilation (i.e., hypercapnia, capillary flow velocity increased relative to normal breathing (i.e., normocapnia). Moreover, different capillary regions showed differing rates of flow-velocity increase, suggesting regional differences in vascular compliance. Spatial variability in blood flow secondary to neural activity, has been observed by several other groups using a variety of different imaging methods [16,17]. Variation in vascular compliance may result from regional differences in arteriovenous diameter, the distribution of the sphincter-pumps that may control hemodynamics [7,8] or spatial reach of cellular signaling mechanisms (back-propagation) that cause dilation of vessels [16] distal to the site of neural activity.

Vascular sensitivity and compliance is also known to vary between subject populations. The aging brain, for instance, undergoes dramatic changes involving global volume, synaptic density, oxygen uptake, and microvascular organization [18-21]. These factors probably affect vascular density and compliance and may contribute to the altered fMRI signal that is observed in older compared to younger adults. These age-related changes most likely exert minimal

effect on neural function *per se*, but may underlie the compromised performance of the neurovascular system [22]. Age-related changes in human cerebrovasculature are likely to exert effects on the coupling between neural activity and hemodynamic response [23-26]. Age-related changes in noise and signal-noise characteristics of fMRI have also been observed [23].

Prior fMRI-BOLD Scaling methodologies

Extensive differences in hemodynamic properties between subjects and between brain regions within subjects suggest that little confidence can be extended to findings of regional or group activation differences during task performance. To overcome some of these limitations of fMRI response, a number of researchers have attempted to characterize regional hemodynamic differences by inducing physiological perturbations that stimulate hemodynamic activity without significantly altering neural activity.

One method has been to induce moderate hypercapnic response by requiring subjects to breathe a CO₂/air mixture. The resulting parameter estimations (of cerebral blood flow) permit scaling of task-induced signal changes [27,28] to account for regional vascular differences. Very recently, studies using hypercapnic methods have used breath hold as a hypercapnic stimulus [29,30].

Empirical demonstration of the equivalence of these two methods has important implications for the study of BOLD physiology as invasive and cumbersome nature of CO₂ makes it intractable for the routine collection of functional neuroimaging data. Subject compliance is significantly compromised by the requirement to breathe CO₂ through a mask prior to every functional imaging study. The problem is compounded for special populations (e.g., psychiatric patients, children and elderly) who are resistant to wearing a gas mask while lying inside the MR scanner. In this study, we demonstrate the functional equivalence of breath hold-induced moderate respiratory hypercapnia and CO₂-induced hypercapnia and validate the fMRI-BOLD signal response after scaling using the breath hold amplitude (BHA) and the square root of the Fourier power in the low frequency band at 0.04Hz which can be defined as the low frequency spectral amplitude (LFSA) that was verified to be significantly free from motor-task interference. Scaling using LFSA and BHA led to a decrease in the variation of BOLD signal response induced by finger-tapping in not only individual subjects but also between subjects.

Methods

Ten healthy volunteers (22 and 38 years of age) with no history of head trauma, neurological disease or hearing disability were scanned. All protocols in this study were approved by the Institutional Review Board, UMDNJ-New Jersey Medical School. Written consent was obtained from all subjects after explanation of the nature and possible consequences of the study.

Rest, breath-hold and finger tapping scans

Subjects performed 3 different tasks during MRI scanning. In one scan, subjects rested while breathing room air. In another scan, subjects performed bilateral finger-tapping for 20 sec followed by rest for 20 sec in three epochs while breathing room air. For the finger tapping task, subjects were instructed to successively touch each finger with the thumb of the fingers' respective hand in a self-paced manner. In a third scan, subjects performed inspirational breath hold for 20 sec followed by normal breathing for 80 sec in three epochs while breathing room air. Subjects were instructed to partially inspire before holding their breath. This did not lead to tensing of the chest similar to a valsalva maneuver and resulted in subjects to perform their

breath hold task for the 20 sec duration without any discomfort towards the end of the breath hold epoch.

CO₂ inhalation scan

To measure similarity of response between the inspirational breath hold paradigm and hypercapnia, four of the subjects performed an additional scan in which they periodically breathed a mixture of 5% CO₂, 20% O₂ and 75% N₂ for 20 sec followed by breathing room air for 80 sec in three epochs. The gas mixture was delivered using a latex inflatable 30L gas-bag (containing the 5% CO₂; Acme Gas Bags, Phoenix AZ) connected to the subjects' gas mask. All subjects practiced breathing 5% CO₂ outside the scanner prior to scanning. A pulse oximeter with infrared sensor was placed on the index finger of the subject (POET 8100, Criticare Systems, Inc., WI, USA) before they were positioned on the scanner gantry. The gas mask, attached to a gas-bag via a plastic hose, was placed over the subject's nose and mouth. Subjects were instructed to rest quietly in the scanner and to breathe normally. During scanning, one of the experimenters (BB) connected and disconnected the gas-bag to the gas mask according to the stimulus protocol.

Imaging parameters

All images were obtained on a Siemens Allegra 3T imaging system equipped with a transmit/receive birdcage radio-frequency head coil. Volunteers were positioned supine on the gantry with the head in a midline location in the coil. To reduce motion artifacts, foam padding was placed between the forehead and the coil. For each subject, echo-planar images were obtained in the axial plane in 6 slices covering the motor cortex using a 64×64 matrix, TR/TE=1 sec/27 msec, FOV=22 cm, slice thickness=7 mm and a bandwidth of 125kHz. The imaging procedure for each volunteer was as follows: sagittal localizer images were first obtained with a conventional gradient echo sequence. The mid sagittal image was used to select the axial slice over the motor cortex for functional imaging. In each subject, 360 echo planar images were obtained for a total scanning time of 6 min. Stimulation task was accomplished by verbal instructions matching the ON/OFF paradigm that was delivered through a microphone and a speaker headphone system worn by the subject.

fMRI data for all experimental runs were preprocessed using AFNI [31]. Reconstructed images were corrected for motion using a rigid-body volume registration. fMRI images in the breath hold and CO₂ scans were registered to the finger tapping scans using 3Dregistration in AFNI [31]. Voxel-wise correlation of signal intensity time course with a boxcar reference function was used to determine activation [32] using a threshold of 0.5 for the correlation coefficient ($p < 0.0001$; after Bonferroni correction for multiple comparisons). Temporal autocorrelation was not accounted for, as no spatial smoothing was preferred due to loss of resolution. For each voxel, the average percent change in the BOLD signal intensity defined as the signal enhancement during the stimulus with respect to the baseline during the pre-stimulus condition was determined from the three stimulus epochs. Voxels that passed the threshold during finger tapping task comprised a mask for further data analysis on a subject-wise basis.

Hemodynamic scaling was accomplished using two methods. In the first method, the signal amplitude during task activation (finger tapping) was scaled using BHA using the relation:

$$S_{sca} = S / (1 + B/B_{ave}) \quad (1)$$

where S_{sca} is the scaled signal change in each voxel during the finger tapping task, S is the unscaled average signal change in each voxel during the finger tapping task, B is the average signal change in each voxel during the breath hold task and B_{ave} is the mean of the signal

change during the breath hold task from all voxels that had significant activation during the finger tapping task from the same subject.

In the second method, the spectral amplitude of the 0.04Hz frequency (LFSA) from the Fourier power spectrum of the fMRI signal time series during the finger tapping task was used to scale the task related fMRI signal using the relation:

$$F_{sca} = F / (1 + P / P_{ave}) \quad (2)$$

Where F_{sca} is the scaled signal in each voxel during the finger tapping task, F is the unscaled average signal change in each voxel during the finger tapping task, P is the square root of the low frequency Fourier power in each voxel during the motor task and P_{ave} is the mean of the square root of the low frequency Fourier power from the region of activation during the motor task.

The ratio of the breath hold signal and the average breath hold signal in the motor cortex and the ratio of the Fourier power of the 0.04Hz frequency and the average of the 0.04Hz frequency in the motor cortex represented in equation (1) and (2) would eliminate large values of the ratio as opposed to a simple division [28], because it provides means to normalize even when the difference between task-related activity (i.e., percent-change from baseline) and hypercapnia-related activity is minimal. In cases where this difference approximated to 0 (~ 0), simple division would result in very large numbers ($\sim \infty$). Another problem raised by simple division is that voxels from regions known to be little-affected by hypercapnia (for example white matter where hypercapnia change has been shown to be significantly lesser than gray matter; e.g., with change $< 1\%$) would result in very large activation change after hemodynamic scaling. In a recent study, the breath hold signal was used to minimize the group variation in working memory tasks [30]. However, a threshold limit for the breath hold signal of 0.5 % was chosen based on the assumption that breath hold signal threshold within the limits of 0.25 to 1% had little effect on the working memory signal variability in specific regions of interest in the brain. The scaling relation as represented by equation (1) and (2) which indicates the ratio of the LFSA or BHA from their average response in the motor cortex would hold good for any value of the hypercapnia response and throughout all brain regions without any limitation. According to equations (1) and (2), the BOLD signal will be scaled by a maximum factor of 2 if the pixel value equals the ROI average.

Results

Comparison of breath hold and CO₂-related signal change

An increase in the intensity of the T2* weighted images during breath hold is associated with a cerebral vasodilatory response, as a result of hypercapnia, leading to an increase in CBF. To test this within our partial inspirational breath hold paradigm, we measured the change in the spatio-temporal extent of BOLD signal under hypercapnic conditions in all subjects breathing a gas mixture of 5% CO₂, 20% O₂ and 75% N₂. As shown in Fig 1a, activated regions during the breath hold task corresponded to activated regions during CO₂ inhalation. Each voxel typically took 12-16 seconds to register an increase in BOLD signal intensity after the onset of breath hold (Fig 1b). A similar delay was observed when the subject breathed 5% CO₂ (Fig. 1c). However, the recovery of the BOLD signal to baseline levels was relatively faster during breath hold when compared to 5% CO₂ inhalation. Blood oxygen saturation monitored by pulse oximetry did not change significantly during breath hold or during mild hypercapnia induced by CO₂ inhalation in any subject. The average resting blood oxygen saturation for all human subjects was 97-98% and did not change significantly either during the breath hold or CO₂

administration. A significant correlation was observed between the BOLD signal intensity change in corresponding voxels during the breath hold task and hypercapnia induced by CO₂ (Fig 1d). This indicates that the partial inspirational breath hold related increase in T2* intensity resulted from a hypercapnic-vasodilatory response. Our results are similar to those observed by Kastrup et al., [33] where a 5% CO₂ inhalation was compared with an expirational breath hold paradigm.

Power spectral characteristics of the resting state fMRI-BOLD signal and its relationship to breath hold response amplitude

Fig 2a shows the Power spectrum from the gray matter voxel of a typical subject. To assess the relationship between the amplitude of the breath hold response and the FFT power of the breath hold task frequency, the breath hold amplitude and the respective Fourier power of the breath hold-task frequency was correlated. As depicted in Fig 2b from a typical subject, a high correlation of 0.61 was observed between the breath hold BOLD response amplitude and its FFT power. Further, the square root of the FFT power of the breath hold signal correlated better ($cc=0.78$) with the breath hold signal amplitude (Fig 2c). A similar trend was observed over all subjects.

The dependence of specific frequencies that may represent respiration induced change in the temporal pattern of BOLD signal was investigated by correlating the FFT power of specific frequency bands from the finger tapping task with the corresponding BOLD signal amplitude during the breath hold task for all voxels in the brain. As depicted in Fig 3a-c in the very low frequency region, the 0.01, 0.03 and 0.04 Hz frequencies in the power spectrum showed a good linear correlation with BHA with the best linear correlation occurring at 0.04 Hz (Fig 3c). However, the frequency corresponding to the breathing rate at 0.3 Hz did not show a good correlation with the breath hold signal (Fig 3d).

While frequencies at 0.01 and 0.03 Hz significantly overlapped with finger-tapping task (Fig 4b,d,e), the 0.04 Hz frequency had no significant interference with the finger tapping task frequency (Fig 4f) in addition to having the best spatial correlation with the BHA map (compare Figs 4a&f). Further, as shown by the power spectrum of the finger-tapping task, the dominant frequency of the finger-tapping task was centered at 0.01 to 0.25 Hz (Fig 4c). Since the power of the 0.04 Hz frequency was out of the way from the dominant frequency of the task in addition to having the best correlation with BHA over all subjects it was best suited as the scaling factor to normalize motor-task induced responses. This would significantly minimize the confound from the motor task-induced BOLD hemodynamic response frequency.

Motor task related fMRI-BOLD response and hemodynamic amplitude scaling

To diminish the contribution of the vasodilatory response to the resultant fMRI signals during finger tapping, the amplitude was scaled using corresponding parameters measured during breath hold in the corresponding voxels using equation (1) to validate the method where the task-induced fMRI response was scaled using LFSA of the motor task induced signal using equation (2). Mean hemodynamically scaled BOLD intensity change in the significant voxels from the motor cortex across all subjects during finger tapping was $4.35 \pm 1.75\%$ using BHA and $4.36 \pm 1.65\%$ using the LFSA. Both were significantly different from the un-scaled signal change of $7.11 \pm 3.30\%$ during finger tapping on a subject-wise basis ($P < 0.0005$; paired t-test). Fig 5 shows typical activation maps of the percent change in BOLD signal in response to a finger-tapping task from three typical subjects prior to amplitude scaling and after scaling using RFSA and BHA. Percent change in BOLD signal intensity appeared spatially uniform after amplitude scaling using both RFSA and BHA.

The properties of scaling with RFSA was compared with BHA by analyzing the shape of the distribution of the BOLD signal change in response to the finger tapping task. Fig 6a-c shows the motor task-induced BOLD signal distributions from six typical subjects before and after amplitude scaling with LFSA or BHA. As observed from the typical distributions of the BOLD signal intensities during finger-tapping, hemodynamic amplitude scaling shifted the median BOLD response to lower values in addition to reducing the standard deviation within subjects from both methods (also see Table 1). To distinguish the effect of scaling on a subject-wise basis, histograms of the BOLD signal response to finger-tapping from three typical subjects prior to and after hemodynamic scaling with LFSA or BHA is shown in Fig 6d-f. It can be observed that the distributions of the BOLD signal change in response to finger tapping after scaling with RFSA and BHA are comparable. Histograms of the scaled BOLD response with LFSA and BHA were tested for similarity on a subject wise basis using the two-sample Kolmogorov-Smirnov test. No significant difference was observed between the LFSA or BHA-scaled distributions for a significance threshold of $p < 0.05$ between LFSA and BHA over all subjects with the exception of subject-8 (Table 1), (critical value for the K-S test statistic, D was $1.36/\sqrt{36} = 0.227$, for $p < 0.05$). The group average of the un-scaled BOLD response change during the finger tapping task was significantly larger than the scaled response (Table 1). The group variation computed as the ratio of the SD/mean was 0.46 for the unscaled, 0.39 and 0.35 for the BHA and LFSA-scaled responses respectively.

Functional activation maps purporting to show networks of neural activity may reflect, to some extent, maps of relative vascular sensitivity. To test this hypothesis in motor cortex we obtained the relative spatial locations of task-induced and breath hold-induced activity and plotted voxels whose activity was significantly correlated with the finger tapping tasks (ROI-wise corrected). These results showed substantial overlap in the activation induced by these two tasks. Indeed, fully 50-60% of the voxels that showed significant finger tapping-related activity also showed significant breath hold related activity. Among the spatially overlapping pixels active during finger tapping and breath hold, a significant correlation was observed in the amplitude of BOLD signal change over all subjects. The correlation between the finger-tapping and breath hold response from six typical subjects is shown in Fig 7.

To determine how vascular sensitivity affects MR signal distribution, relative to neural activity, we plotted correlations between finger tapping-related and breath hold-related activity from the same spatial voxels at a number of different statistical thresholds. As shown in Fig 8, finger tapping and breath hold-related correlation increases with increasing statistical threshold. This result lends credence to the hypothesis that those brain regions showing the greatest task-related activity are those where vascular sensitivity is the greatest.

Discussion

The partial inspirational breath hold paradigm was validated using parallel CO₂ inhalation studies in four of the six subjects. A high correlation between the BOLD signal change during the partial inspirational breath hold and 5% CO₂ breathing tasks validated the similarity of the partial inspirational breath hold paradigm with gas-induced hypercapnia. Our results were similar to those observed by Kastrup et al., [33] where they have compared 5% CO₂ inhalation with an expirational breath hold paradigm. The partial inspirational breath hold task is simple to perform and significantly eliminates the discomfort during the last few moments of the breath hold task. Such a paradigm would lead to better subject compliance and can be easily performed by special populations (e.g., psychiatric patients, children and elderly).

Equivalence of LFSA and BHA as hemodynamic scaling factors

Our results imply the existence of a population of voxels with high-amplitude signal that exceeded statistical threshold more as a result of proximity to vascular activity (e.g., voxels

located close to large draining veins) than proximity to neural activity. These were the regions that responded with a high amplitude response during the breath hold task. The correlation of LFSA with BHA during the finger-tapping task indicates a similar sensitivity of the breath hold BOLD amplitude and the Fourier power of the 0.04Hz frequency to arterial CO₂ changes (Fig 3c). In an earlier study, Birn et al., [34] have shown that the 0.03 Hz frequency correlated with variation in respiration per time (RVT) in subjects breathing spontaneously during an fMRI scan. Though the breathing pattern was not monitored in our study to show evidence that the 0.04 Hz frequency band was indeed related to RVT, the proximity of the 0.04 Hz frequency band to the RVT and the fact that the RVT frequency can vary from subject to subject indicates that the 0.04 Hz frequency may most probably contain a large component of the respiration variation as the subjects in our study were also breathing spontaneously.

Prior to hemodynamic scaling, we observed an approximately $7.11 \pm 3.30\%$ mean signal change and a broad distribution of activated voxels across the sensorimotor cortex during a finger-tapping task (Table 1 and Fig 6a). After hemodynamic amplitude scaling with LFSA, the finger tapping-induced BOLD signal response reduced to $4.36 \pm 1.65\%$ (Table 1 and Fig 6b). Hemodynamic scaling using BHA reduced the finger tapping response to a similar level of $4.35 \pm 1.75\%$ (Table 1 and Fig 6c). Histograms of the scaled BOLD response with LFSA and BHA were tested for similarity on a subject wise basis using the two-sample Kolmogorov-Smirnov test. As shown in Table 1, no significant difference was observed between the LFSA or BHA-scaled distributions for a significance threshold of $p < 0.05$ between LFSA and BHA over 90% of the subjects. These results indicate that hemodynamic scaling with LFSA was equivalent to BHA. Scaling with either LFSA or BHA reduced the group variation in the BOLD signal response. A reduction of 15% and 23% in the group variation was observed after scaling with BHA and LFSA respectively.

The correlation between LFSA and BHA suggests that vascular sensitivity exerts a similar effect on the MR-T2* signal during breath hold and physiological noise arising from respiration. This is further supported by the observation of systematic increases in finger-tapping task and breath hold signal relationships with increases in statistical threshold and the correlation of the extent of the breath hold signal (Fig 7). These relationships appear to have a distinct anatomical distribution, as reflected in the overlap between task- and breath hold induced motor cortex activity. That is, some regions evince signal with greater contribution from vasculature than others.

Determination of the effect of vascular sensitivity on the MR-T2* signal distribution

We determined the correlations between finger tap-related and breath hold-related activity at a number of different statistical thresholds. As can be seen in Fig 8, increases in the correlation threshold eliminated mostly voxels showing small signal change, and a few voxels with large signal change during both the finger-tapping and breath hold tasks. This manipulation increased the correlation between the BOLD signal response amplitude between the two tasks as observed by an increase in the correlation in the scatter plots (Fig 8). This result indicates that regions showing the greatest activity during finger tapping may be the most sensitive to vascular changes, but not necessarily those showing the greatest task sensitivity. Such results suggest the necessity of applying correction methods that account for intrinsic vascular changes prior to interpretation of task-related MR signal amplitudes.

Minimization of vascular sensitivity from neural response

Since vascular anatomy varies considerably between brain regions, spatial variation in BOLD signal change during task activation could reflect either cerebrovascular or cerebrometabolic activity [35]. Assuming that oxygen consumption remains stable during mild hypercapnia [36], hypercapnic stress such as breathing CO₂ or a breath hold task may be used to normalize

functional activation by minimizing the contribution from the cerebrovascular component of BOLD signal change [27-30].

The hemodynamic scaling method using LFSA demonstrated here provides a method to minimize non-neural signal change, thus providing a better validity between-population comparisons without the additional requirement of a hypercapnic stimulus such as breath hold. Delivery of mild hypercapnic stimulus in humans require breathing 5% CO₂ from a source and requires the subject to wear a face-mask. Breath-hold has been used as an alternate method to achieve hypercapnia [37-39]. Though it is less invasive and less problematic in patients for whom co-operation regarding task performance is an issue [40], breath hold has the advantage of inducing a hypercapnic stimulation without a mask with excellent patient tolerance [41]. However, subject compliance issue during the breath hold task can be a concern for special populations such as children [42].

In conclusion, the breath hold-validated LFSA based correction as demonstrated in this study can be used to scale task-induced signal change and may be observed and quantified across brain regions to reduce the influence of subject compliance, brain regional and population differences in brain activation studies using BOLD-fMRI.

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Figure 1.

Time-courses of a representative voxel from the motor cortex in a typical subject during, **a)** bilateral finger tapping, **b)** breath hold, **c)** moderate hypercapnia with 5% CO₂, and **d)** scatter plot of voxels showing significant percent change ($cc = 0.5$) during breath hold with the percent change in the corresponding voxels during moderate hypercapnia (5% CO₂, 20% O₂ and 75% N₂) from a typical subject. A significant correlation between hypercapnia with 5% CO₂ and breath-hold was observed in all 3 subjects tested. The stimulus paradigm consisted of three "ON" cycles or task alternating with periods of rest "OFF" ('REF' in the figure).

Figure 2.

a) Fourier power spectrum from the gray matter voxel in the absence of any task, **b)** correlation of BHA versus the FFT power at the frequency of the breath hold task and **c)** correlation of BHA versus the square root of the FFT power (spectral amplitude) at the frequency of the breath hold task. The square root of the FFT power (spectral amplitude) correlates better than the absolute FFT power. Each data point in the scatter plots indicate a single voxel obtained from the whole brain in a typical subject.

Figure 3.

Correlation between BHA versus the LFSA at specific frequencies during the finger tapping task, **a)** 0.01 Hz, **b)** 0.03 Hz, **c)** 0.04 Hz and **d)** 0.3 Hz respectively. The correlation at 0.04 Hz was the best without significant overlap from the task-induced signal change. Data are from the whole brain from a typical subject. LFSA at 0.04Hz showed a high correlation with BHA over all subjects.

Figure 4.

Spatial correlation of LFSA during finger-tapping versus BHA from the same subject shown in figure 3. **a)** BHA, **b)** Finger-tapping **c)** LFSA at 0.01 Hz, **d)** LFSA at 0.03 Hz and **e)** LFSA at 0.04 Hz. The 0.01 and 0.03 Hz frequencies seem to be contaminated by the finger tapping task frequency. LFSA at 0.04 Hz, has a good spatial correlation with BHA with minimal contamination from the task signal (finger-tapping).

Figure 5.

Activation maps in response to the motor task (finger-tapping) superimposed on the BOLD signal maps in three typical subjects. **a)** unscaled, **b)** scaled using LFSA and **c)** scaled using BHA. Colors indicate the percent change from baseline and were statistically determined by cross correlating a reference function as described in the methods section. A threshold of 0.5 for the correlation coefficient ($P < 0.0001$) after a Bonferroni correction for multiple comparisons was considered for significance. Voxels with very high percent change in the unscaled images seem to be scaled to values comparable to spatially adjoining voxels after scaling with LFSA or BHA.

Figure 6.

Frequency distribution of the percent change in fMRI-BOLD signal intensity from the voxels that passed the statistical threshold of $cc \geq 0.5$ ($P < 0.0001$) in six typical subjects during the finger-tapping task. **a)** unscaled, **b)** scaled using LFSA and **c)** scaled using BHA.

Figure 7.

Scatter plots of the BOLD signal change during finger tapping task versus the BHA in the same voxels in six typical subjects. A high correlation is observed in the signal change during the two experimental paradigms. A statistical threshold of $cc \geq 0.5$ ($P < 0.0001$) was used to obtain the activated voxels.

Figure 8.

Scatter plots of the BOLD signal change during finger tapping task versus the BHA in the same voxels in six typical subjects with different correlation thresholds ($cc \geq 0.40$ to 0.65). Voxels with high signal change in the finger-tapping task are better correlated with the corresponding voxels from the breath hold task.

Mean percent change in fMRI-BOLD signal during a finger-tapping task and BHA in the activated motor cortex over all subjects.

Table 1

	Average % change BOLD (breath hold)	Mean±SD of the task-induced BOLD signal response in the motor cortex (% change)			* Kolmogorov-Smirnov test statistic (D)
		Un-scaled	Scaled (BHA)	Scaled (LFSA)	
Subject 1	7.48	7.35±5.22	4.18±3.41	3.74±2.77	0.08
Subject 2	8.85	12.06±8.60	5.90±2.13	6.32±5.28	0.20
Subject 3	6.72	6.40±2.84	3.30±1.36	3.30±1.36	0.13
Subject 4	8.62	10.39±5.55	6.33±2.97	5.43±2.56	0.21
Subject 5	10.51	9.46±5.30	6.59±4.39	6.10±5.41	0.18
Subject 6	7.76	9.19±8.24	5.64±5.94	6.17±8.03	0.10
Subject 7	2.66	2.09±4.22	1.77 ±0.77	1.57 ±0.80	0.09
Subject 8	3.72	2.77±3.30	2.08± 0.82	3.24± 1.43	0.23
Subject 9	6.99	7.28±11.66	4.45± 4.81	4.83± 3.09	0.10
Subject 10	4.25	4.15±3.45	3.45±3.81	3.75±2.20	0.09
Group ave	6.76 ±2.49	7.11± 3.30	4.35 ±1.75	4.36± 1.65	

* Kolmogorov-Smirnov test comparison of BHA-scaled and LFSA-scaled distributions.