

Reducing Vascular Variability of fMRI Data across Aging Populations Using a Breathholding Task

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Abstract: The magnitude and shape of blood oxygen level-dependent (BOLD) responses in functional MRI (fMRI) studies vary across brain regions, subjects, and populations. This variability may be secondary to neural activity or vasculature differences, thus complicating interpretations of BOLD signal changes in fMRI experiments. We compare the BOLD responses to neural activity and a vascular challenge and test a method to dissociate these influences in 26 younger subjects (ages 18–36) and 24 older subjects (ages 51–78). Each subject performed a visuomotor saccade task (a vascular response to neural activity) and a breathholding task (vascular dilation induced by hypercapnia) during separate runs in the same scanning session. For the saccade task, signal magnitude showed a significant decrease with aging in FEF, SEF, and V1, and a delayed time-to-peak with aging in V1. The signal magnitudes from the saccade and hypercapnia tasks showed significant linear regressions within subjects and across individuals and populations. These two tasks had weaker, but sometimes significant linear regressions for time-to-peak and coherence phase measures. The significant magnitude decrease with aging in V1 remained after dividing the saccade task magnitude by the hypercapnia task magnitude, implying that the signal decrease is neural in origin. These findings may lead to a method to identify vascular reactivity-induced differences in the BOLD response across populations and the development of methods to account for the influence of these vasculature differences in a simple, noninvasive manner. *Hum Brain Mapp* 28:846–859, 2007. © 2006 Wiley-Liss, Inc.

Key words: hypercapnia; aging; CBF; CBV; BOLD

INTRODUCTION

Functional MRI (fMRI) has the potential to be a powerful tool in clinical neuroscience. It is noninvasive, can be safely repeated many times in an individual, and provides data from the entire brain with a spatial and temporal resolution of millimeters and seconds, respectively. However, before fMRI becomes a reliable clinical tool, several important data interpretation issues must be considered. The blood oxygen level-dependent (BOLD) signal, frequently used as a dependent measure in fMRI experiments, is an indirect measurement of neural activity.

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Increases in neural activity, via a still incompletely characterized mechanism, cause an increase in cerebral blood flow (CBF), leading to an increase in oxygenated hemoglobin reaching a local brain region. This in turn leads to a decrease in the local concentration of paramagnetic, deoxygenated hemoglobin and a BOLD signal increase [Buxton, 2002]. Therefore, an increase in BOLD signal is usually interpreted as an increase in neural activity. However, any factor that alters vascular reactivity or the coupling between neural activity and the vascular response will influence the BOLD signal, and complicate interpretations of BOLD signal [D'Esposito et al., 2003].

The factors that can influence the BOLD signal independent of changes in neural activity are diverse. These include individual differences in the size and location of veins [Bandettini and Wong, 1997; Cohen et al., 2004], extracranial arterial disease [Hamzei et al., 2003; Rother et al., 2002], small vessel cerebral disease [Pineiro et al., 2002], pulse or respiration differences [Dagli et al., 1999; Hu et al., 1995], hematocrit concentrations [Levin et al., 2001], and baseline CBF [Cohen et al., 2002]. Several studies have also revealed that medications and food intake can affect the BOLD signal. For example, alcohol consumption increases CBF and the resting BOLD signal and may decrease the BOLD difference between rest and active conditions [Levin et al., 1998]. Recent lipid consumption also decreases BOLD signal changes [Noseworthy et al., 2003]. A vasoconstrictor, such as caffeine, decreases the resting BOLD signal, but the response to neuronal activity remains the same, so that the BOLD contrast is increased [Mulderink et al., 2002]. A later study showed that the magnitude of the BOLD contrast changes in response to caffeine was highly variable across subjects [Laurienti et al., 2003].

These factors greatly complicate the interpretation of differences in the BOLD signal when comparing across populations, especially those that might have different vascular systems, such as younger and older study groups [for reviews, see D'Esposito et al., 2003; Gazzaley and D'Esposito 2005]. One example of how vascular alterations in aging might affect the BOLD signal is described by Hajdu et al. [1990]. They demonstrate that at a constant pressure, the vessel wall diameter, distensibility, and amount of smooth muscle and elastin in pial arterioles in rats decrease with age. The BOLD signal is strongly linked to changes in CBF, which is controlled by the compliancy of the vessel wall. If there is a decrease in the range of vascular compliance with aging, then the BOLD signal will exhibit a narrower range in an older population. One PET study showed a global decrease in CBF response to hypercapnia with healthy aging and hypothesized that this was caused by sclerotic changes in arteries with aging [Ito et al., 2002].

Accordingly, human fMRI studies have shown BOLD signal differences between younger and older populations during simple tasks when the underlying neural activity is

expected to be almost identical. The rise time of the BOLD signal in response to a simple motor task increases slightly with age [Taoka et al., 1998]. Older subjects also exhibit a smaller spatial extent of significant activity and lower signal-to-noise during a simple visuomotor task [D'Esposito et al., 1999], as well as decreased signal magnitude in a visual cortex, but not a motor cortex [Buckner et al., 2000]. This decrease in magnitude might be partially explained by more voxels with a negative BOLD signal, which could either be due to a neuronal or vascular change [Aizenstein et al., 2004]. Behzadi and Liu [2005] attempted to include these age-related changes in their BOLD signal mode.

High BOLD signal variability is apparent even in healthy young controls that are not taking any medications. Several empirical studies examined signal variability using a simple visuomotor task where the neural responses were expected to be similar across subjects. The BOLD signal was shown to vary across healthy, young volunteers [Aguirre et al., 1998] and across regions in the same subject [Miezin et al., 2000]. Although the BOLD signal is highly variable across subjects, the signal time-to-peak was correlated across most combinations of primary visual and motor cortices and frontal and supplementary eye fields during a visuomotor saccade task [Handwerker et al., 2004].

Since there are so many nonneural factors, even in healthy adults, that may affect the BOLD signal, it is not practical to control for all these factors. This is a more significant problem for comparisons across populations when the nonneural factors might confound the results. It is more practical to develop a normalization method that adjusts the data for any nonneural variation regardless of its source. In this study, we use hypercapnia, an increase in blood P_{CO_2} , achieved with a breathholding task, to further the development of a method for normalizing the BOLD signal for vascular differences. During hypercapnia, CBF increases diffusely, resulting in an increase in the ratio of oxygenated hemoglobin to deoxygenated hemoglobin, and thus a robust, global increase in the BOLD signal [Kety and Schmidt, 1948; Kwong et al., 1992; Li et al., 1999]. Decreases in pH due to hypercapnia may cause the CBF increase [Kontos et al., 1977]. One theory on how pH affects CBF is that a change in pH alters the affinity that K^+ and Ca^{2+} have to the same binding sites. With a lower pH there is more K^+ bound and the extracellular K^+ concentration decreases, causing less membrane depolarization on vascular smooth muscle and less vasoconstriction [Madden, 1993]. Unlike increases in BOLD signal due to neural activity, which are relatively localized around the site of neural activity, the changes in pH and thus the increases in BOLD signal magnitude are global. Since neural activity and hypercapnia both increase BOLD through a CBF increase, the BOLD signal changes from each should be similarly affected by variations in vascular mechanics.

Bandettini and Wong [1997] collected BOLD signal data during both a hypercapnia and a motor task. While the primary purpose of their study was to identify voxels that corresponded to draining veins, they also showed a linear relationship between signal magnitudes obtained during hypercapnia and the motor task. Hamezei et al. [2003] showed a link between a decreased BOLD signal during hypercapnia and a signal decrease during a motor task in patients with cranial artery disease. Another study using a similar task showed that the magnitudes of the BOLD response during both a motor task and hypercapnia decrease with aging [Riecker et al., 2003]. These findings imply that, without a vascular measure, it is impossible to confirm that a BOLD signal decrease is caused by neural and not vascular changes.

Several studies have attempted to use hypercapnia to calibrate the BOLD signal and minimize vascular effects to extract a more direct measure of neural activity from fMRI data. These include simultaneous measurement of BOLD and CBF for single [Davis et al., 1998] or multiple [Hoge et al., 1999] concentrations of inhaled CO₂ to derive an estimate of the cerebral metabolic rate of oxygen (CMRO₂), which is more closely linked to neuronal activity. An fMRI study with rats measured BOLD, CBF, and also cerebral blood volume (CBV) to estimate CMRO₂ [Wu et al., 2002]. Although these studies yielded measures that may more accurately reflect neural activity than the BOLD signal, they have significant limitations. They all require CBF measurements, which have a lower temporal resolution and often fewer slices of data than BOLD-weighted images [Silva and Kim, 2003]. Also, accurate measures of CBV require a pulse sequence with a large time-to-repetition (TR) for vascular space occupancy (VASO) imaging [Lu et al., 2003] or the invasive injection of a contrast agent. In addition, these estimations of CMRO₂ require a significant amount of scan time to get an accurate normalization factor. An ideal calibration measure would require only limited scanner time so that it could be coupled with the primary study of interest. Ideally, such a task could also be easily and safely performed by participants and preferably not require extra equipment such as masks for CO₂ breathing.

It is unlikely that it will be practical in most fMRI studies to obtain accurate measures of CMRO₂ given these constraints, but it might be possible to adjust for some vascular differences across regions and populations using other methods. For example, Cohen et al. [2004] derived a method to adjust the BOLD signal for the influence of baseline CBV levels. Using standard equations for the BOLD signal, given a constant MRI echo time, they show that the change in BOLD signal in a voxel is a function of fixed parameters multiplied by the change in venous hemoglobin saturation. The fixed parameters are measures of baseline venous blood volume in a voxel, tissue relaxation times, baseline intra- and extravascular signal magnitudes,

and the MRI echo time. Within a given voxel, all these parameters are constant regardless of the mechanism that causes changes in hemoglobin saturation. Therefore, if BOLD signal changes to neural activity and hypercapnia are both recorded and the hypercapnia task causes global CBF changes, dividing the changes in BOLD signal from the two tasks normalizes for the baseline CBV and baseline intrinsic signal parameters, leaving a relative measure of change in venous hemoglobin saturation. They used a sequential finger movement task and 5% CO₂ breathing as their two conditions and demonstrated the stability of this normalization method across several magnet field strengths and pulse sequences [Cohen et al., 2004]. One limitation of their interpretation of their model is that it includes several fixed parameters, such as baseline measures of the BOLD signal magnitudes, in addition to baseline CBV. These values might also change across populations due to aging, disease, or medication. Assuming these values are constant within each voxel in each subject, the equations in the Cohen et al. study also show that dividing by the hypercapnic BOLD response normalizes these values. It is probable that baseline CBV is the dominant value normalized using hypercapnia, but the work of Hamzei et al. [2003] and Riecker et al. [2003] show changes in the hypercapnic response that probably are not baseline CBV changes.

In the present study, we further explore this normalization method by collecting data from a larger population of younger and older subjects using a 20-s breathholding task to induce hypercapnia. Although there is less control over the level of hypercapnia in this task as compared to inhalation of CO₂, one goal of this study was to find a practical way to remove vascular variability from BOLD data. Breathholding requires minimal additional equipment and all subjects are able to easily complete this task. Twenty seconds of breathholding induces sufficient hypercapnia to produce a robust BOLD signal increase [Liu et al., 2002]. Breathholding produces similar responses to CO₂ breathing [Kastrup et al., 2001] and multiple publications have BOLD responses to hypercapnia using breathholding [Kastrup et al., 1999; Kwong et al., 1995; Stillman et al., 1995]. By comparing the BOLD response generated by hypercapnia to that produced by a visuomotor saccade task across voxels and regions in younger and older subjects, our aim was to determine if a linear relationship between these task responses exists across subjects of different ages, and thus assess if breathholding-induced hypercapnia represents a useful normalization method for BOLD fMRI data.

MATERIALS AND METHODS

Subjects

Fifty subjects with no history of neurological, psychiatric, vascular, or respiratory diseases participated in the experiment. Twenty-six of these subjects were in the “younger”

group (ages 18–36, mean: 24, 14 male) and 24 subjects were in the “older” group (ages 51–78, mean: 64, 10 male). Informed consent was obtained from each volunteer.

Data Collection

MRI data

T2*-weighted echo-planar images (EPI) were acquired with a 4 T Varian INOVA MR scanner (Palo Alto, CA) and a TEM send and receive RF head coil (MR Instruments, Minneapolis, MN) using a two-shot gradient echo EPI sequence (22.4-cm² field of view with a 64 × 64 matrix size resulting in an in-plane resolution of 3.5 × 3.5-mm for each 5-mm slice, echo time = 28-ms, flip angle = 20°). For 13 young and 13 older subjects, 18 axial slices were acquired with a time repeat (TR) = 2-s and a 0.5-mm gap between slices, and for 13 young and 11 older subjects, 10 oblique slices, parallel to the calcarine sulcus, were acquired with a 1.1-s TR and a 1-mm gap between slices. The 2-s TR data were collected as part of other studies [Gazzaley et al., 2005a,b]. EPI distortions between the two shots due to motion or other phase changes were decreased using navigator echoes [Ehman and Felmlee, 1989; Kim et al., 1996]. Adjacent half *k*-space shots were interpolated to decrease the sampling interval to half of the total TR [Noll, 2000]. Three half *k*-space segments from each interpolated TR by combining the uninterpolated middle segment with the bilinear interpolation of the two flanking segments. Therefore, volumes were 0.5*TR-s apart, but each volume used data collected over 1.5*TR-s. High-resolution MP-FLASH 3D T1-weighted scans (256 × 256 × 128 voxels with 0.875 × 0.875 × 1.4-mm³ resolution) were acquired for anatomical localization.

Visuomotor saccade task

Subjects fixated their eyes on a small cross. When a 20-Hz flickering, oval checkerboard appeared for 200 ms, the cross moved up or down in the annulus and subjects quickly pressed buttons with both hands and moved their eyes to the new cross location. The cross remained in its new location until the next stimulus. There were 18–22 s intertrial intervals (ITI) for the 2-s TR and 19.8–24.2 s ITIs for the 1.1-s TR. For the 2-s TR, each subject completed 20 trials during a single run. For the 1.1-s TR, each subject completed 40 trials divided into two identical runs.

Hypercapnia task

Subjects saw a 30-s countdown and visual instructions to breath in or out approximately every 3-s. After the final exhale, subjects held their breath during a 20-s countdown. The subjects breathed out before the holding period both to decrease the amount of oxygen remaining in their lungs and because it was observed that some subjects moved their heads while trying to take a very deep breath if they

were permitted to breath in immediately before the holding period. This was repeated seven times and ended with 30-s of breathing. The instructions always filled the same area of the screen with almost constant luminance, so there was no or minimal task-correlated change in visual stimulation. Three young and three older subjects did not have the 30-s breathing period at the end. Visual stimuli were used since it was possible to make a fairly constant amount of visual stimulation. If auditory cues were used, subjects would need to hear “out” or “in” every 3-s or would need to memorize auditory cues representing each task state. Considering that this normalization procedure might be used in populations with cognitive deficits, memorizing complex cues might be challenging. The confound of potentially increased baseline activity in visual cortex is balanced by studying data in both visual and motor brain regions. Respiratory rates were monitored in real time to ensure task compliance using a respiratory belt transducer (AD Instruments, Charlotte, NC).

Data Analysis

All data were motion-corrected using SPM2 (Wellcome Department of Imaging Neuroscience, London, UK). No significant task-correlated motion was observed during the saccade task in the subjects used in this study and no subjects were removed due to excessive motion. The data were neither spatially smoothed nor normalized because that would increase the statistical dependence of data across voxels and bias this study towards finding correlations across voxels. The data have a small amount of inherent spatial smoothing since raw MRI data contain some smoothing and motion correction adds more smoothing. For each subject the frontal eye fields (FEF), supplementary eye fields (SEF), primary motor cortex (M1), and primary visual cortex (V1) were anatomically defined based on gyral and sulcal landmarks defined in the Duvernoy Human Brain Atlas [1999]. FEF followed part of the precentral sulcus, SEF was medial and included the paracentral sulcus, M1 followed the central sulcus, and V1 followed the calcarine sulcus. The anatomical masks may contain other active brain regions. For example, since the V1 region of interest (ROI) follows the calcarine sulcus, it may also contain parts of V2, V3, and V4.

Significantly active voxels for the saccade task and for the hypercapnia task were identified using a General Linear Model (GLM) analysis with a Fourier basis set in SPM2 [Bullmore et al., 1996; Josephs et al., 1997]. For the saccade task, the temporal derivative of the three spatial and three rotational motion correction parameters were included as covariates of no interest. Since this study compares data across voxels rather than mapping which regions are active in a task, a more liberal threshold, corrected for multiple comparisons, was used to allow more data to be included in subsequent analyses. The statistical

threshold was set at $P < (4/\text{total \# of voxels in the four anatomical masks})$. The total number of voxels in each subjects' anatomical mask was between 601 ($P < 0.0067$) and 1,461 ($P < 0.0027$) voxels with a mean of 936 ($P < 0.0043$) voxels per subject.

Time series from both tasks were extracted from the voxels that were significantly active during the saccade task. The saccade task data were highpass-filtered at 1/128 Hz. The hypercapnia data were bandpass-filtered between 1/128 and 1/5 Hz. The data were scaled to percent change from the mean and then averaged across trials to get a single trial time course for each task from each voxel (Fig. 1). For the saccade task, the trial data were interpolated to 1/4 the TR and the percent change was the maximum value in the trial averaged time course and the time-to-peak was the time to that value. For the hypercapnia task, the percent change was the mean difference between the maximum and minimum three values in the trial averaged time series. While averaging across three values might decrease some of the noise compared to using only one value, it is an arbitrary choice, but other choices gave almost identical results. The percent change values were also calculated averaging across 2–6 maximum and minimum values, using only the maximum values, and taking the minimum values from the first 20 s and maximum values from the last 30 s in the time series. In all these cases, collapsed across subjects and in almost every region in all subjects, the alternate breath-holding percent change values had a strong linear correlation ($R^2 > 0.9$) to the values used in this study. Because the difference between the minimum and maximum values were used, the percent change values are larger than us-

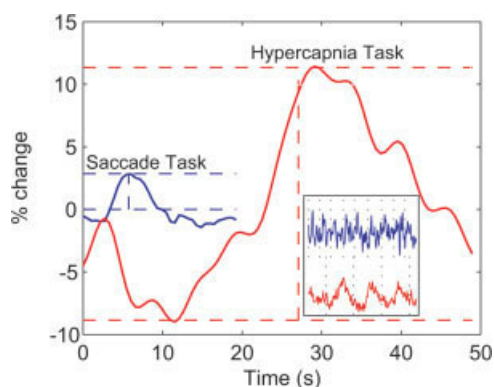


Figure 1.

Trial averaged time series for the hypercapnia and visuomotor saccade tasks from a single FEF voxel in a young subject. The horizontal dashed lines mark the values used to calculate percent change for the task and the vertical lines are the time-to-peak and rise-time estimates; 0% change is the mean of the time-series. The inset plots show the first 192 s from each task with the dashed vertical lines showing the beginning of each trial.

ually seen in fMRI studies. Still, 20-s of hypercapnia creates a larger response than most neural tasks. The rise-time for the hypercapnia task used interpolated data, as did the saccade task, and was defined as the first time point where the response was greater than 90% of the hypercapnia percent change. Figure 1 shows a trial averaged time series from each task. Horizontal dashed lines mark the values used to calculate percent change and the vertical lines mark the time-to-peak estimates.

Coherence was also used to measure phase delays in each task [Sun et al., 2004, 2005]. The seed was a cluster of the 11 voxels in the SEF containing the maximum F ratio from the saccade task and the 10 contiguous voxels with the largest F ratios. The 11 time series from these voxels were averaged to generate the time series that would be compared to other voxels in the brain. The same voxels were used to generate the seed time series for a coherence analysis of the hypercapnia data. The analyses averaged over the frequencies from 0–0.15 Hz. This frequency range includes the dominant frequencies for both tasks. The coherence phase data were compared across the saccade and hypercapnia tasks only for the voxels that showed significant coherence in both tasks.

Significantly active voxels from the GLM analyses were identified using SPM2. Some file manipulations used functions in *fmristat* by K.J. Worsley (www.math.mcgill.ca/keith/fmristat). The coherence analyses used in-house code written by F.T. Sun [Sun et al., 2004, 2005]. Most processing after significantly active voxels were identified used in-house code written in MATLAB (MathWorks, Natick, MA). All linear regressions that used data from multiple subjects were done in Stata (College Station, TX). For all these regressions, unless otherwise stated, the data from the saccade task were treated as the dependent variable and data from the hypercapnia task were the independent variables. Other independent variables were sometimes added including age, population, and TR. Since values within each subject are less independent than values across subjects, Stata's robust variance estimates for clustered data were used for all regressions with voxel data from multiple subjects [Williams, 2000]. In these analyses, robust variance tended to be larger than variance, making it less likely to find significant differences. Since this study attempts to identify any possible causes of signal variation, false-negative values are worse than false-positive values. Therefore, all regression statistics with P -values less than 0.05 are reported without corrections for multiple comparisons.

RESULTS

Extent and Magnitude of BOLD Signal

Extent: voxel counts

Table I shows the mean number of activated voxels across the four ROIs in the saccade task for younger and

TABLE I. Voxel counts across ROIs

		Percent of significant voxels in ROI masks: saccade task	Percent of significant voxels active in the saccade task that were also active during hypercapnia	No. of voxels in the anatomical masks
1.1-s TR, 40 Trials	Young	17.8 ± 8.7	77.4 ± 15.5	686 ± 112
	Old	17.5 ± 9.1	87.3 ± 10.9	743 ± 108
2-s TR, 20 Trials	Young	13.9 ± 7.3 ^a	76.4 ± 15.9	1071 ± 157 ^b
	Old	5.6 ± 3.6 ^a	74.5 ± 8.6	969 ± 140 ^b

ROI, region of interest.

^a $P = 0.0006$.

^b $P = 0.0471$.

older subjects. All values show mean plus the standard error. To account for different anatomical mask sizes across subjects, mean voxel counts were calculated by determining the percent of activated voxels across all anatomical masks in each subject. The data were also separated by whether they were collected with a TR = 1.1 s or TR = 2 s since a different number of trials were collected for each TR.

The percentage of significantly active voxels across ROIs was less in the older group as compared to the younger group in the saccade task (Table I). This difference was only statistically significant in the TR = 2 s data. For the TR = 2 s data, the raw number of significantly active voxels also was less in the older group, suggesting that the data presented in Table I are not due to variation in mask sizes since the subjects with larger masks and a constant raw volume of activity would have a smaller percent of significant voxels. Table II shows the percentage of significantly active voxels separated by ROI. The decrease in the older group in the saccade task is significant in all four ROIs for TR = 2.

Although the liberal threshold allows more voxels to be included in the signal magnitude calculations, since the liberal threshold used to identify significant activity might affect differences in voxel counts, all voxel counts for the saccade task were reanalyzed using a $P < 0.05$ threshold

Bonferroni-corrected for the number of voxels in the four ROIs. While all voxel counts were lower, there were still no significant differences with aging for the 1.1-s TR subjects and there was still a significant difference for the 2-s TR subjects ($P = 0.002$) in Table I. In Table II, all significant or nonsignificant differences with aging were the same, except there was no longer a significant decrease in M1 for the 2-s TR ($P = 0.055$) and there was a significant decrease in V1 for the 1.1-s TR ($P = 0.027$).

Table I also shows that a high percentage of voxels that were significantly active for the saccade task were also significantly active during the hypercapnia task. While this percentage changed across ROIs, there were no systematic differences across ROIs.

Magnitude: percent signal change

Mean percent signal change values were calculated by finding the percent signal change in each voxel and taking the mean across the regions and populations of interest. The mean voxel percent change values for the saccade task across all subjects were 1.7% in FEF, 1.8% in M1, 1.4% in SEF, and 1.9% in V1. SEF was significantly different from the other ROIs ($P < 10^{-4}$), and FEF was significantly different from V1 ($P \leq 0.002$). The mean voxel percent change values from the hypercapnia task across voxels across sub-

TABLE II. Voxel counts for each ROI

		Percent of significant voxels in masks: saccade task			
		FEF	M1	SEF	V1
1.1-s TR, 40 Trials	Young	15.1 ± 8.5	11.1 ± 7.4	16.9 ± 10.6	39.2 ± 14.3
	Old	16.9 ± 11.9	15.2 ± 8.6	14.1 ± 9.7	28.0 ± 18.7
2-s TR, 20 Trials	Young	11.4 ± 7.6 ^a	10.4 ± 5.7 ^b	13.9 ± 9.6 ^c	19.6 ± 10.8 ^d
	Old	4.2 ± 3.5 ^a	5.5 ± 4.3 ^b	5.3 ± 4.5 ^c	7.3 ± 4.4 ^d

^a $P = 0.0024$.

^b $P = 0.0107$.

^c $P = 0.0037$.

^d $P = 0.0004$.

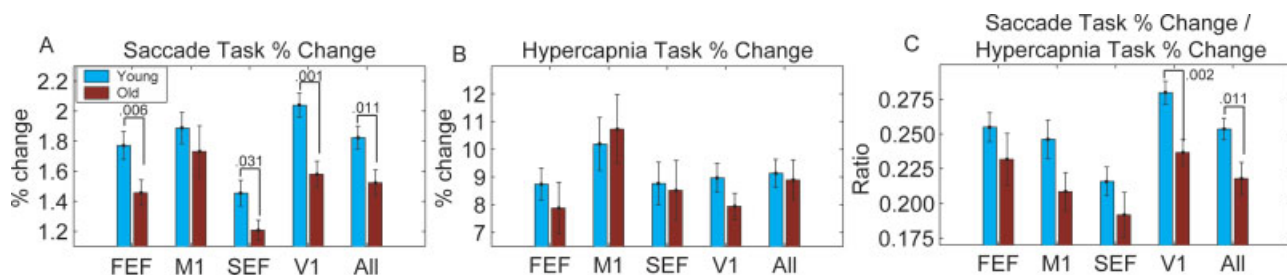


Figure 2.

Bar graphs of regions and populations. **A,B:** Mean percent change across voxels in all subjects during the saccade task and the hypercapnia task, respectively. **C:** Mean of the percent change during the saccade task divided by the percent change during the hyper-

capnia task in each voxel. The error bars show the robust standard error clustered by subject. The *P*-values are shown above significant differences and were calculated from regressions that compared across populations and included a dummy variable for TR.

jects were 8.4% in FEF, 10.4% in M1, 8.7% in SEF, and 8.7% in V1. M1 was significantly different from the other ROIs ($P < 0.023$).

Figure 2A,B shows the mean signal percent change for the peak magnitude during the saccade and hypercapnia tasks for each population in each ROI and collapsed across ROIs. Collapsed across ROIs, a significant decrease in magnitude was found from younger to older subjects during the saccade task but not during the hypercapnia task. Within individual ROIs, there were significant differences across populations in FEF, SEF, and V1 during the saccade task. Figure 3A–D shows that the distributions of the mean signal percent change values across groups are almost identical.

In addition to comparing percent change in younger vs. older subjects, we used regression analyses to examine percent change vs. age. Since each TR had a different number of trials and slices, TR was also included in the regressions as a dummy variable. Percent change during the saccade task was significantly correlated with age in FEF ($P = 0.01$), SEF ($P = 0.042$), V1 ($P = 0.002$), and across all regions ($P = 0.005$). Percent change during the hypercapnia task was not significantly correlated with age. Although there were a different number of trials and slices for each TR, neither the percent change during the saccade task nor the percent change during the hypercapnia task significantly changed with TR. This was true for the young and old subjects grouped together and for each group analyzed separately. This demonstrates that the results were not biased by the data from one sampling rate.

BOLD Signal Relationships for Saccade vs. Hypercapnia Tasks

Linear regression analysis was used to compare the percent signal change by voxel of the saccade task vs. the hypercapnia task. The selected voxels were significantly active during the saccade task and all comparisons across tasks used the same voxels for each task.

Collapsed across ROIs

There was a significant linear regression between activity in the saccade task vs. hypercapnia with voxels from all ROIs and clustered by subject ($P < 10^{-26}$, $R^2 = 0.566$, slope = 0.0959, and the intercept = 0.843). When subjects were divided into younger and older populations, the slope of the regression for younger subjects was 0.100 and 0.087 for older subjects. Neither the slope nor intercept differences across the populations were significant. There was also a significant linear regression in most individual subjects. Figure 4 shows examples of these regressions from four younger and four older subjects. Forty-eight of the 50 subjects showed significant linear regressions of signal

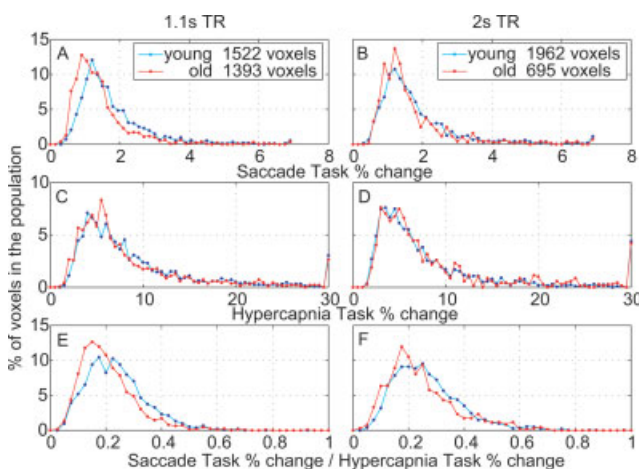


Figure 3.

A,B: Histograms of percent signal change during the saccade task. **C,D:** Percent signal change during the hypercapnia task. **E,F:** The ratio, by voxel of the percent signal changes of the saccade task divided by the hypercapnia task. This includes data from all subjects and all anatomical masks. Histograms **A,C,E** use a 1.1-s TR and **B,D,F** use a 2-s TR. Since each population had a different number of subjects and a different raw number of significantly active voxels, the y-axis was scaled to percent of voxels in that population.

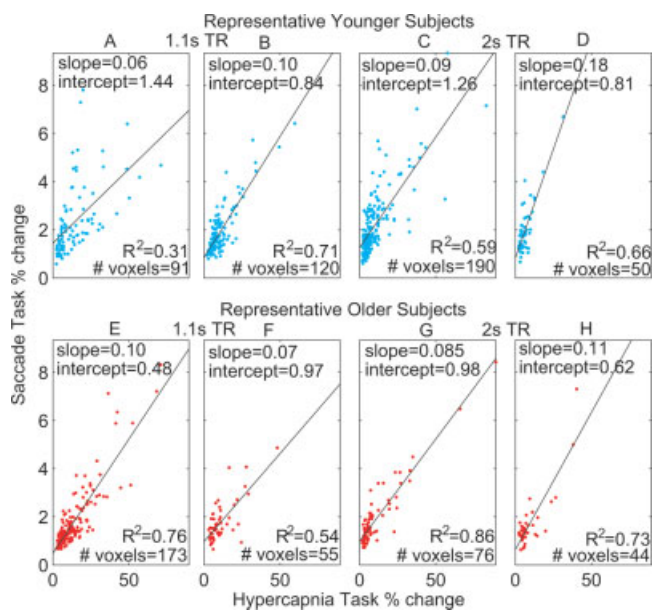


Figure 4.

Percent change by voxel from the saccade vs. the hypercapnia tasks from eight representative subjects. Each plot uses significantly active voxels during the saccade task from FEF, M1, SEF, and V1 in a single subject. Each point is data from a single voxel. All linear regressions are statistically significant. This shows that the magnitude of the response to hypercapnia can predict the magnitude of the response to a neuronal task within each subject.

change in the saccade vs. hypercapnic tasks ($P < 0.01$). The mean and standard error of the R^2 values across the 48 subjects were 0.555 and 0.175, respectively. Forty-five subjects showed significant correlations with $P < 0.0005$. The two subjects who did not show a significant linear correlation ($P < 0.01$) were both older subjects with a 2-s TR and the second and third fewest significant active voxels used in the regression (21 and 22 voxels). Figure 3E,F shows that the distributions of saccade task percent change divided by the hypercapnia task percent change by voxel were similar in the younger and older populations.

Some voxels with very high percent signal change values were examined for artifacts that might cause outliers. The voxels examined had similar time course shapes as the rest of the voxels, except that the percent signal change was much higher. While the similar shapes imply the signal changes were caused by physiological sources, they could be markers of draining veins. For the comparisons tested, removing voxels with extremely large percent signal change did not alter the significance of the correlations.

The significant, positive bias of the intercept was noted across all subjects and within many subpopulations. When only voxels where both the breathholding and saccade tasks were used in the correlations, the magnitude of the

intercept decreased, but it was still significantly greater than 0. A nonzero intercept can be observed in at least one other article [Bandettini and Wong, 1997].

Additional regressions included a dummy variable for TR to test if TR affected the slope or intercept of the saccade vs. hypercapnia tasks percent change correlations. TR did not affect the slope of this regression in either young or old subjects or across both populations, but did affect the intercept of the regression in older subjects only ($P = 0.031$).

Individual ROIs

For each ROI collapsed across subjects, either within each population or across both populations, there was a significant linear regression of BOLD signal across the two tasks ($P < 10^{-5}$). Figure 5 shows voxels from the four ROIs for all young and old subjects. The slope was significantly different between younger and older subjects only in SEF ($P < 0.005$). The slope difference was approximately the same size in FEF, but the difference was not significant since there was a larger robust standard error. For the voxels in V1, TR significantly affected the slope ($P < 0.001$) and the intercept ($P = 0.027$) of the regression. TR did not affect the regression in the other three ROIs. Across all subjects, the slopes and intercepts were also significantly different between pairs of regions. The slope in SEF was significantly different from FEF ($P < 0.03$) and M1 ($P < 0.004$). The intercept for V1 was significantly different from FEF ($P < 0.05$), M1 ($P < 0.002$), and SEF ($P < 0.003$). Correlation coefficients were larger for the younger subjects.

Figure 2C shows the mean signal change for each population and ROI if the saccade task magnitude was divided by the hypercapnia task magnitude for each voxel. In this case, there were still significant differences in V1 and collapsed across all ROIs. This ratio decreased from younger to older subjects in the other three ROIs, but the decrease was not statistically significant.

Temporal Dynamics of BOLD Signal across Groups

Figure 6 shows the mean time-to-peak values from the saccade task for each ROI and population. The plots are separated by TR since TR significantly affected time-to-peak values ($P < 0.03$). Collapsed across all ROIs and both TRs, younger subjects have shorter mean time-to-peak values ($P = 0.001$). Figure 6 shows the specific regions where the difference is statistically significant. Linear regressions between the saccade and hypercapnia task for temporal data were examined using two different metrics. Within the same voxels derived from the percent signal change analysis, time-to-peak during the saccade task was compared to rise-time during the hypercapnia task. Phase delays calculated from a coherence analysis using signifi-

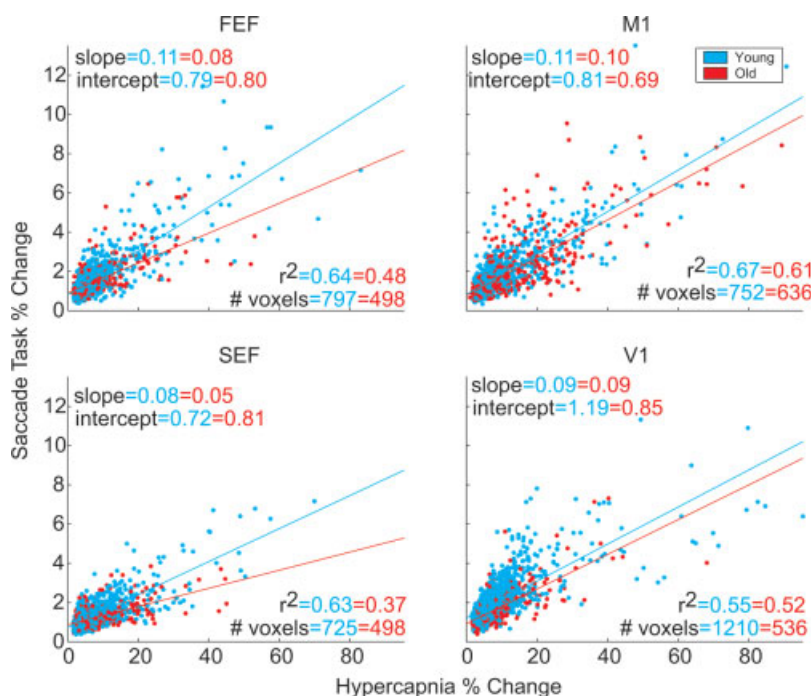


Figure 5. Percent change by voxel from the saccade vs. the hypercapnia tasks for all young and old subjects. Each plot shows data from all subjects for each ROI. All eight regressions are significant. There was a statistically significant slope difference between younger and older subjects in SEF ($P < 0.005$).

cantly active voxels based on an SEF seed during the saccade and hypercapnia task were also compared. Since TR significantly affected temporal measurements, all regressions were calculated for each TR separately or included TR as a dummy variable. For both of these metrics, while there are sometimes significant linear regressions, the fits are very noisy, and there are no clear patterns across subjects.

The time-to-peak and rise-time values from the saccade and hypercapnia task, when clustered across all subjects, were significantly correlated ($P = 0.027$ and $R^2 = 0.0128$). When this regression was calculated for each subject separately, only 14 out of 50 subjects showed a significant correlation ($P < 0.05$). For these 14 subjects, the mean and standard deviation R^2 values across subjects were 0.089 and 0.069, respectively.

The coherence phase data from the saccade and hypercapnia tasks, when clustered across all subjects, were linearly correlated ($P < 0.0001$ and $R^2 = 0.0243$). When calculating this regression for each subject separately, only the subjects who had at least five significantly active voxels from the coherence analysis were used. Only 13 of the 44 remaining subjects showed a significant linear correlation ($P < 0.05$). In these 13 subjects, the mean and standard deviation R^2 values were 0.150 and 0.161, respectively.

For both time-to-peak and coherence phase calculations the number of subjects with significant correlations and their R^2 values are much lower than the correlations using percent change. Although there may be a temporal correlation between the saccade and hypercapnia tasks, the large

amount of variability, both with the small number of subjects showing a linear correlation and with the small R^2 values, limits the predictive value of this correlation.

DISCUSSION

Neural activity, vasculature, and the physical limitations of fMRI all cause variation in the BOLD signal. Most

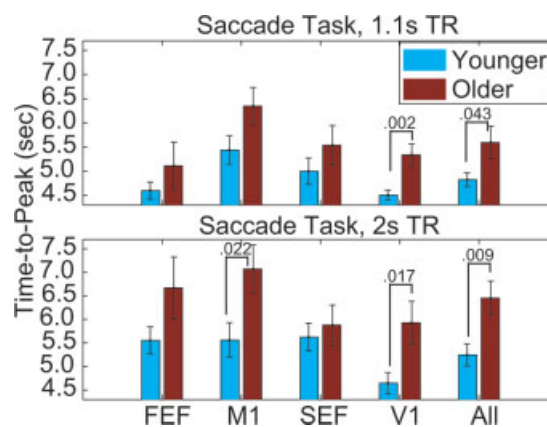


Figure 6. Time-to-peak by ROI for younger and older subjects. Each bar is the mean time-to-peak value across all voxels in the ROI from all subjects in the specified population. Error bars show the robust standard error. When there is a significant difference between younger and older subjects, the P -value is noted.

researchers who use fMRI want to identify variation caused only by neural activity and consider other sources of variation unwanted noise. Vascular variation is especially significant for studies across populations since it might create a systemic bias of the BOLD signal. We collected BOLD data while inducing localized neural activity changes using a simple visuomotor saccade task and identified systematic variations of the BOLD signal between younger and older subjects. We also collected BOLD data while inducing global vascular changes using a hypercapnia task and, using linear regressions, showed how this removes a significant portion of the vascular variability from the BOLD signal. We propose that a method, like the one described here, can be used for vascular normalization. As shown by Cohen et al. [2004], this normalization procedure removes some vascular noise caused by baseline CBV variability across voxels. It may also correct for other sources of vascular variability. In either case, removing any source of nonneural variability decreases noise and increases the likelihood of finding significant results.

Several studies, sometimes with contradictory results, have examined younger and older volunteers while measuring signal-to-noise ratio (SNR), counts of voxels that were significantly active, and temporal and magnitude changes of BOLD responses. We also used these metrics with a large population of younger and older subjects to look for differences and determine which of the previously published results were replicable and how they were affected by our vascular correction.

Spatial Extent of Activation

We showed that younger subjects had a larger mean number of significantly active voxels than older subjects. The difference was statistically significant for the 2-s TR, but not the 1.1-s TR data (Table I). The decreased spatial extent of activation with aging replicates findings in several other studies [Buckner et al., 2000; D'Esposito et al., 1999; Huettel et al., 2001]. While there is a decrease in the mean spatial extent with aging, Table I also shows large standard errors across subjects within each population. This is important to note since, although there is a mean decrease, there is large overlap across populations and there is sometimes more variation across subjects within a population than across populations.

One possible cause of the decrease in spatial extent is decreased SNR in older subjects caused by either increased noise or decreased signal magnitude [D'Esposito et al., 1999; Huettel et al., 2001]. The subjects whose data were collected with a 1.1-s TR had twice as many trials as the 2-s TR subjects. With an increased sampling rate and an increased number of trials, the spatial extent of activation increased in both younger and older subjects and the difference in extent between the two populations decreased.

If the true spatial extent of neural activation was similar in both populations, then an SNR decrease could explain the decrease in spatial extent of the BOLD data. If SNR is a cause of the decreased spatial extent of activity, then collecting additional data and decreasing the TR should decrease spatial variability.

Saccade Task Signal Magnitudes

As shown in past studies, during a neural task, such as the saccade task, there are significant signal magnitude changes across regions and magnitude decreases from younger to older subjects. Our findings replicate the BOLD signal decrease in V1 with aging [Buckner et al., 2000]. We also show that, even though a significant portion of the signal variability can be explained using the percent signal change during a hypercapnia task, there is still a significant difference in V1 (Fig. 2C). Assuming the hypercapnia task accounts for all vascular variability, then the observed BOLD signal decrease has neural origins. A similar study showed a decrease in M1 with aging both during a finger-tapping task and during hypercapnia [Riecker et al., 2003]. We did not replicate this finding in M1. The decrease in M1 during the visuomotor saccade task was not significant and the signal increased with aging in the hypercapnia task in M1 (Fig. 2). There are several possible reasons for this difference. Riecker et al. [2003] used the single voxel with the highest *t*-value rather than an average across multiple voxels to calculate their signal changes and voxels with the largest signal changes, and, especially at 1.5 T compared to higher field strengths, the largest *t*-values, potentially contain large draining veins [Bandettini and Wong, 1997]. In addition, their study included only 15 subjects, while the present study included 47 subjects. It is possible that the observed difference was due to sampling bias in a small number of voxels from a small population. Finally, Riecker et al. [2003] used a paced finger-tapping task. Due to saturation effects, their longer duration motor task may increase population-specific differences to statistically significant levels. If this final point is the cause of the difference, it might imply that population-specific biases could be a larger issue in fMRI studies with long duration blocks of activity rather than short duration events.

Aizenstein et al. [2004] proposed an alternative explanation for the signal decreases with age. They noted that many of the past studies found significantly active voxels regardless of hemodynamic response function (HRF) shape and averaged the time series across voxels before calculating the percent signal change. They showed that this method might include negative BOLD responses that would decrease the mean magnitude of the positive responses. Their data showed that older subjects had more voxels with negative responses. For our study, significantly active voxels were identified using a shape-independent method.

To test if the Aizenstein et al. hypothesis was correct, we divided our voxels into positive and negative responses, depending on the magnitudes of the maximum and minimum time points in each voxel's HRF. We replicated the first part of the Aizenstein et al. [2004] finding and showed that older subjects had fewer voxels with positive time series than young subjects. Across the four ROIs, the percentages, with standard error, of positive voxels were, for the 1.1-s TR, $90 \pm 6\%$ in younger subjects, and $78 \pm 11\%$, in older subjects. For the 2-s TR, they were $74 \pm 9\%$ in younger subjects and $58 \pm 11\%$ in older subjects. However, this did not account for our signal magnitude decrease with aging. Our results, shown in Figure 2A, calculated peak magnitude from each voxel separately and then averaged the peak values. In this case, since the magnitudes were never negative, this alone could not account for our signal decrease. We also recalculated our signal magnitudes for each population using the same metrics as Aizenstein et al. [2004] along with a Bonferroni-corrected threshold of $P < 0.05$. The significant decrease with aging in V1 remained when we reran statistical tests using the maximum F value from each subject ($P < 0.0001$), the mean of the positive voxels ($P < 0.03$), and the mean of the voxels that were significant with a Bonferroni-corrected threshold ($P < 0.05$). In addition, Figure 3A,B shows similar distributions of the signal magnitudes across voxels in young and old subjects, and Huettel et al. [2001] also show a similar distribution. We show that older subjects are skewed to slightly lower magnitudes, while Huettel et al. [2001] shows that young subjects have slightly lower magnitudes. There were minimal changes in the shapes of these histograms when only positive voxels were used. If older subjects had a different mean value because they had a subpopulation of voxels with a distinctly different pattern of activity, the shapes of the histograms for the younger and older subjects would not be similar. These findings show that averaging time series data across voxels is not the cause of observed signal decreases across populations.

Timing Differences

This study showed that signal time-to-peak for the saccade task is longer in the older population. This finding complements the results of Taoka et al. [1998], who showed a positive correlation between the time to reach half the maximum magnitude and age in the precentral sulcus. A time-to-peak bias between populations can significantly affect fMRI study results since a temporal misestimate of the hemodynamic responses can cause systemic errors in magnitude estimates [Handwerker et al., 2004].

Using Hypercapnia for Vascular Normalization

The data from the hypercapnia task were used to examine whether it accounted for any of the variability in the

saccade task. Since the two tasks are completely independent and the hypercapnia task does not selectively engage neuronal activity in any of the four ROIs in a task-correlated manner, similarities between the data from the two tasks must result from similarities of vascular reactivity and not neural activity.

This study showed an extremely strong linear correlation between the magnitudes, by voxel, of the saccade and hypercapnia tasks. The correlation was observed in 48 of 50 individuals. Two other studies used CO₂ breathing to show this relationship [Bandettini and Wong, 1997; Cohen et al., 2004]. Our study shows the result in a much larger population that also includes older subjects. In addition, our study demonstrated that this same correlation of magnitudes could be reliably induced using breathholding-induced hypercapnia rather than CO₂ inhalation. Because this relationship exists, it should be possible to divide the magnitude of a neural task from the magnitude of the hypercapnia task, by voxel, to normalize for vascular variability. One should note that the correlation coefficients were lower for the older volunteers (Fig. 5). With the earlier observation of lower SNR affecting the spatial extent of activation, this might point to requiring more subjects of data to obtain accurate signal measurements across noisier populations.

The data in this article includes an example where vascular normalization adds information that would significantly alter the interpretation of results. Figure 2A shows significant decreases with aging for percent signal change during the saccade task in FEF, SEF, and V1. With only this data, it is impossible to state whether these decreases are neural in origin. Figure 2C shows that the significant magnitude decrease remained in V1, but not FEF and SEF after magnitude normalization using hypercapnia. These results imply that the V1 decrease with aging is neural in origin, while the FEF and SEF decreases might have some vascular origins. Without a valid vascular normalization procedure it would be impossible to distinguish the signal decreases in these two regions.

The hypercapnia task was not a good marker of temporal vascular variability. While both time-to-peak and coherence phase measures sometimes showed a significant linear regression between the saccade and hypercapnia tasks, the coefficients of determination were small and the data showed large variability across subjects. It is possible that there is a temporal correlation across the two tasks, but the chosen metrics did not measure the correct aspects of the signals. Time-to-peak gives values relative to the start of each trial. It is a commonly used measure, but it is very sensitive to noise since it identifies a specific time point that crosses a threshold. For this reason, coherence phase was also used. This measure gave temporal lags relative to a seed in the SEF and used the whole time series, and therefore is less sensitive to noise and the specific choice of TR. Coherence showed more significant linear regres-

sions between the saccade and hypercapnia tasks, but, since most subjects did not exhibit significant linear regressions, it also may not be a useful normalization measure. Other ROIs and seed selection methods for the coherence analysis were also tested but none showed more consistent results. Since neither of these measures showed a clear temporal relationship between the two tasks, even if a relationship exists, breathholding-based hypercapnia is probably not a reliable temporal normalization measure.

Other Sources of Variability

The hypercapnia task accounted for a significant amount of the magnitude variation in the saccade task, but there were still very large magnitude, temporal, and spatial extent variability between subjects and populations. Some of this variation could be caused by task compliance. For example, the subjects made manual responses to flickering checkerboards, but we did not record eye movements within the scanner to document compliance on this aspect of the task. As with many fMRI studies, we did not control for caffeine intake or timing of a preceding meal, both of which may increase variability across subjects [Laurienti et al., 2003; Noseworthy et al., 2003]. Variations in blood pressure may even affect the BOLD signal [Wang et al., 2004].

Another source of variability during the hypercapnia task is chest movement. There is a slight shift in the magnetic field susceptibilities that depend on the size of the chest and how much it moves. Since each subject alternated paced breathing with breathholding, chest motion was correlated with the task. This will affect the signal magnitude and slightly shift the location of voxels, but it should affect the BOLD signal with no temporal lag. Figure 1 shows that while the subject starts breathholding at time 0, the peak is temporally delayed, so this artifact does not significantly affect the percent signal change. Some of the smaller signal changes due to chest movements may be visible in Figure 1 with breathing cycles starting at 20, 27, 33, 39, and 45 s. Since this artifact might also shift voxel locations slightly, this might be of concern when comparing it to other neural tasks or to an anatomical image. CO₂ inhalation studies will not have this artifact, so additional studies that compare CO₂ inhalation to breathholding, such as has been done in one existing study, would be useful [Kastrup et al., 2001]. Kastrup et al. [2001] showed that BOLD signal magnitudes from CO₂ inhalation and breathholding are similar, but the goal of their study was not specifically to examine image shifts caused by additional chest movement during a breathholding task.

The image shift due to chest movement may significantly confound spatial activity maps from breathholding. This is especially true if one population has a smaller average chest size or takes larger breaths. Greater chest dis-

placement may produce more significantly active voxels due to voxel shift motion. This study avoided this confound by using the saccade task to identify significant voxels. Table I shows that most voxels that were significantly active during the saccade task were also active during the hypercapnia task, so it is reasonable not to require a specific test for significance for the hypercapnia task.

Finally, there is the question of how much the correlations between the signal magnitudes in the saccade and hypercapnia tasks are purely a function of blood vessel sizes. Additional studies comparing perfusion or blood volume images to BOLD data from a neuronal task would provide information regarding this issue. Nevertheless, while the signal change in the hypercapnia task is definitely a function of baseline CBV, the hypercapnia task may also invoke mechanisms relating to vascular reactivity, which are not part of a static vascular map. This is especially relevant in clinical populations where there might be local vascular compliance changes.

One source of variation was avoided in this study by not using an estimated shape of the hemodynamic response. In studies that use a constant hemodynamic response estimation for all voxels, variations in the time-to-peak can affect the magnitude estimates [Handwerker et al., 2004]. By using a frequency-based analysis to find time series of any shape that are correlated to the task frequency and then calculating the signal change from the mean, this study removed a potential confound.

Limitations of Vascular Normalization by Hypercapnia

One goal of this study was to determine a method to normalize for vascular variability across subjects and populations. Hypercapnia is one approach, but dividing by a single run of breathholding data may not be a perfect solution, since it is still based on MRI data, which contains additional noise. However, since hypercapnia was able to explain a significant and large portion of signal variability, it removes more noise than it adds. Also, without additional research, it is impossible to determine how well the BOLD response to hypercapnia models all the vascular elements of the BOLD response to neural activity, not just baseline CBV, and whether simply dividing the two task magnitudes gives an accurate measure of relative neural activity. Additional research should compare the BOLD responses to neural activity and hypercapnia using “gold standard” measures. This could be done using direct and consistent neuronal stimulation of a region while BOLD data is being collected under exogenously altered vascular states. Designs that could provide this gold standard are being developed [Kennerley et al., 2004; Logothetis et al., 2001]. With tasks such as hypercapnia added to these designs, it will be possible to identify a normalization

method to remove vascular variability that could be used in noninvasive human studies.

CONCLUSION

This study measured changes in the BOLD signal that occur with aging and attempted to develop a normalization measure for vascular variability across subjects and populations. During a visuomotor saccade task, older subjects had lower signal magnitudes and longer time-to-peak measures in all four ROIs (significant when collapsed across all ROIs and for magnitudes in FEF, SEF, and V1 and time-to-peak in M1 and V1). The data collected during the hypercapnia task accounted for a significant amount of the BOLD signal variability observed during the saccade task. Also, the magnitude percent signal changes during the two tasks showed significant linear regressions across all subjects and individually in 48 of 50 subjects. When the signal magnitude from the saccade task was divided by the magnitude from the hypercapnia task, the decrease in aging in V1 remained, implying it is neural in origin. Thus, hypercapnia induced through 20 s of breathholding accounts for a portion of the vascular variability. Importantly, it is a task that can be added to most experimental protocols and it is simple for subjects to perform. Further research is necessary to develop the optimal normalization procedure to account for vascular variability between different populations.

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REFERENCES

- Aguirre GK, Zarahn E, D'Esposito M (1998): The variability of human, BOLD hemodynamic responses. *Neuroimage* 8:360–369.
- Aizenstein HJ, Clark KA, Butters MA, Cochran J, Stenger VA, Meltzer CC, Reynolds CF, Carter CS (2004): The BOLD hemodynamic response in healthy aging. *J Cogn Neurosci* 16:786–793.
- Bandettini PA, Wong EC (1997): A hypercapnia-based normalization method for improved spatial localization of human brain activation with fMRI. *NMR Biomed* 10:197–203.
- Behzadi Y, Liu TT (2005): An arteriolar compliance model of the cerebral blood flow response to neural stimulus. *Neuroimage* 25:1100–1111.
- Buckner RL, Snyder AZ, Sanders AL, Raichle ME, Morris JC (2000): Functional brain imaging of young, nondemented, and demented older adults. *J Cogn Neurosci* 12(Suppl 2):24–34.
- Bullmore E, Brammer M, Williams SC, Rabe-Hesketh S, Janot N, David A, Mellers J, Howard R, Sham P (1996): Statistical methods of estimation and inference for functional MR image analysis. *Magn Reson Med* 35:261–277.
- Buxton RB (2002): Introduction to functional magnetic resonance imaging: principles and techniques. New York: Cambridge University Press.
- Cohen ER, Ugurbil K, Kim SG (2002): Effect of basal conditions on the magnitude and dynamics of the blood oxygenation level-dependent fMRI response. *J Cereb Blood Flow Metab* 22:1042–1053.
- Cohen ER, Rostrup E, Sidaros K, Lund TE, Paulson OB, Ugurbil K, Kim SG (2004): Hypercapnic normalization of BOLD fMRI: comparison across field strengths and pulse sequences. *Neuroimage* 23:613–624.
- Dagli MS, Ingeholm JE, Haxby JV (1999): Localization of cardiac-induced signal change in fMRI. *Neuroimage* 9:407–415.
- Davis TL, Kwong KK, Weisskoff RM, Rosen BR (1998): Calibrated functional MRI: mapping the dynamics of oxidative metabolism. *Proc Natl Acad Sci U S A* 95:1834–1039.
- D'Esposito M, Zarahn E, Aguirre GK, Rypma B (1999): The effect of normal aging on the coupling of neural activity to the bold hemodynamic response. *Neuroimage* 10:6–14.
- D'Esposito M, Deouell LY, Gazzaley A (2003): Alterations in the BOLD fMRI signal with ageing and disease: a challenge for neuroimaging. *Nat Rev Neurosci* 4:863–872.
- Duvernoy HM (1999): The human brain surface, blood supply, and three-dimensional sectional anatomy. New York: Springer.
- Ehman RL, Felmlee JP (1989): Adaptive technique for high-definition MR imaging of moving structures. *Radiology* 173:255–263.
- Gazzaley A, D'Esposito M (2005): BOLD functional MRI and cognitive aging. In: Cabeza R, Nyberg L, Park D, editors. *Cognitive neuroscience of aging*. New York: Oxford University Press. p 107–131.
- Gazzaley A, Cooney JW, McEvoy K, Knight RT, D'Esposito M (2005a): Top-down enhancement and suppression of the magnitude and speed of neural activity. *J Cogn Neurosci* 17:507–517.
- Gazzaley A, Cooney JW, Rissman J, D'Esposito M (2005b): Top-down suppression deficit underlies working memory impairment in normal aging. *Nat Neurosci* 8:1298–1300.
- Hajdu MA, Heistad DD, Siems JE, Baumbach GL (1990): Effects of aging on mechanics and composition of cerebral arterioles in rats. *Circ Res* 66:1747–1754.
- Hamzei F, Knab R, Weiller C, Rother J (2003): The influence of extra- and intracranial artery disease on the BOLD signal in fMRI. *Neuroimage* 20:1393–1399.
- Handwerker DA, Ollinger JM, D'Esposito M (2004): Variation of BOLD hemodynamic responses across subjects and brain regions and their effects on statistical analyses. *Neuroimage* 21:1639–1651.
- Hoge RD, Atkinson J, Gill B, Crelier GR, Marrett S, Pike GB (1999): Investigation of BOLD signal dependence on cerebral blood flow and oxygen consumption: the deoxyhemoglobin dilution model. *Magn Reson Med* 42:849–863.
- Hu X, Le TH, Parrish T, Erhard P (1995): Retrospective estimation and correction of physiological fluctuation in functional MRI. *Magn Reson Med* 34:201–212.
- Huettel SA, Singerman JD, McCarthy G (2001): The effects of aging upon the hemodynamic response measured by functional MRI. *Neuroimage* 13:161–175.
- Ito H, Kanno I, Ibaraki M, Hatazawa J (2002): Effect of aging on cerebral vascular response to Paco₂ changes in humans as measured by positron emission tomography. *J Cereb Blood Flow Metab* 22:997–1003.
- Josephs O, Turner R, Friston K (1997): Event-related fMRI. *Hum Brain Mapp* 5:243–248.
- Kastrup A, Kruger G, Glover GH, Neumann-Haefelin T, Moseley ME (1999): Regional variability of cerebral blood oxygenation response to hypercapnia. *Neuroimage* 10:675–681.

- Kastrup A, Kruger G, Neumann-Haefelin T, Moseley ME (2001): Assessment of cerebrovascular reactivity with functional magnetic resonance imaging: comparison of CO₂ and breath holding. *Magn Reson Imag* 19:13–20.
- Kennerley AJ, Berwick J, Martindale J, Johnston D, Chen D, Lefebvre V, Papadakis NY, Zheng YJM (2004): fMRI with simultaneous optical imaging spectroscopy or laser-Doppler flowmetry: investigation of the hemodynamic response function. San Diego: Society For Neuroscience.
- Kety SS, Schmidt CF (1948): The effects of altered arterial tensions of carbon dioxide and oxygen on cerebral blood flow and cerebral oxygen consumption of normal young men. *J Clin Invest* 27:484–492.
- Kim SG, Hu X, Adriany G, Ugurbil K (1996): Fast interleaved echo-planar imaging with navigator: high resolution anatomic and functional images at 5 Tesla. *Magn Reson Med* 35:895–902.
- Kontos HA, Raper AJ, Patterson JL (1977): Analysis of vasoactivity of local pH, PCO₂ and bicarbonate on pial vessels. *Stroke* 8:358–360.
- Kwong KK, Belliveau JW, Chesler DA, Goldberg IE, Weisskoff RM, Poncelet BP, Kennedy DN, Hoppel BE, Cohen MS, Turner R (1992): Dynamic magnetic resonance imaging of human brain activity during primary sensory stimulation. *Proc Natl Acad Sci U S A* 89:5675–5679.
- Kwong KK, Wanke I, Donahue KM, Davis TL, Rosen BR (1995): EPI imaging of global increase of brain MR signal with breath-hold preceded by breathing O₂. *Magn Reson Med* 33:448–452.
- Laurienti PJ, Field AS, Burdette JH, Maldjian JA, Yen YF, Moody DM (2003): Relationship between caffeine-induced changes in resting cerebral perfusion and blood oxygenation level-dependent signal. *AJNR Am J Neuroradiol* 24:1607–1611.
- Levin JM, Ross MH, Mendelson JH, Kaufman MJ, Lange N, Maas LC, Mello NK, Cohen BM, Renshaw PF (1998): Reduction in BOLD fMRI response to primary visual stimulation following alcohol ingestion. *Psychiatry Res* 82:135–146.
- Levin JM, Frederick BB, Ross MH, Fox JF, von Rosenberg HL, Kaufman MJ, Lange N, Mendelson JH, Cohen BM, Renshaw PF (2001): Influence of baseline hematocrit and hemodilution on BOLD fMRI activation. *Magn Reson Imag* 19:1055–1062.
- Li TQ, Kastrup A, Takahashi AM, Moseley ME (1999): Functional MRI of human brain during breath holding by BOLD and FAIR techniques. *Neuroimage* 9:243–249.
- Liu HL, Huang JC, Wu CT, Hsu YY (2002): Detectability of blood oxygenation level-dependent signal changes during short breath hold duration. *Magn Reson Imag* 20:643–648.
- Logothetis NK, Pauls J, Augath M, Trinath T, Oeltermann A (2001): Neurophysiological investigation of the basis of the fMRI signal. *Nature* 412:150–157.
- Lu H, Golay X, Pekar JJ, van Zijl PCM (2003): Functional magnetic resonance Imaging based on changes in vascular space occupancy. *Magn Reson Med* 50:263–274.
- Madden JA (1993): The effect of carbon dioxide on cerebral arteries. *Pharmacol Ther* 59:229–250.
- Miezin FM, Maccotta L, Ollinger JM, Petersen SE, Buckner RL (2000): Characterizing the hemodynamic response: effects of presentation rate, sampling procedure, and the possibility of ordering brain activity based on relative timing. *Neuroimage* 11:735–759.
- Mulderink TA, Gitelman DR, Mesulam MM, Parrish TB (2002): On the use of caffeine as a contrast booster for BOLD fMRI studies. *Neuroimage* 15:37–44.
- Noll DC, Stenger VA, Vazquez AL, Peltier SJ (2000): Spiral scanning in fMRI. In: Moonen CTW, Bandettini PA, editors. *Functional MRI*. New York: Springer. p 149–160.
- Noseworthy MD, Alfonsi J, Bells S (2003): Attenuation of brain BOLD response following lipid ingestion. *Hum Brain Mapp* 20:116–121.
- Pineiro R, Pendlebury S, Johansen-Berg H, Matthews PM (2002): Altered hemodynamic responses in patients after subcortical stroke measured by functional MRI. *Stroke* 33:103–109.
- Riecker A, Grodd W, Klose U, Schulz JB, Groschel K, Erb M, Ackermann H, Kastrup A (2003): Relation between regional functional MRI activation and vascular reactivity to carbon dioxide during normal aging. *J Cereb Blood Flow Metab* 23:565–573.
- Rother J, Knab R, Hamzei F, Fiehler J, Reichenbach JR, Buchel C, Weiller C (2002): Negative dip in BOLD fMRI is caused by blood flow—oxygen consumption uncoupling in humans. *Neuroimage* 15:98–102.
- Silva AC, Kim SG (2003): Perfusion-based functional magnetic resonance imaging. *Concepts Magn Reson A* 16A:16–27.
- Stillman AE, Hu X, Jerosch-Herold M (1995): Functional MRI of brain during breath holding at 4 T. *Magn Reson Imag* 13:893–897.
- Sun FT, Miller LM, D’Esposito M (2004): Measuring interregional functional connectivity using coherence and partial coherence analyses of fMRI data. *Neuroimage* 21:647–658.
- Sun FT, Miller LM, D’Esposito M (2005): Measuring temporal dynamics of functional networks using phase spectrum of fMRI data. *Neuroimage* 28:227–237.
- Taoka T, Iwasaki S, Uchida H, Fukusumi A, Nakagawa H, Kichikawa K, Takayama K, Yoshioka T, Takewa M, Ohishi H (1998): Age correlation of the time lag in signal change on EPI-fMRI. *J Comput Assist Tomogr* 22:514–517.
- Wang R, Qiao M, Foniok T, Kirk D, Vivanco R, Tuor U (2004): Functional magnetic resonance imaging: effect of blood pressure increases and decreases. San Diego: Society for Neuroscience.
- Williams RL (2000): A note on robust variance estimation for cluster-correlated data. *Biometrics* 56:645–646.
- Wu G, Luo F, Li Z, Zhao X, Li SJ (2002): Transient relationships among BOLD, CBV, and CBF changes in rat brain as detected by functional MRI. *Magn Reson Med* 48:987–993.