Neural Mechanisms of Age-Related Slowing: The $\Delta$CBF/$\Delta$CMRO$_2$ Ratio Mediates Age-Differences in BOLD Signal and Human Performance

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The precise mechanisms that give rise to the blood-oxygen-level-dependent (BOLD) activation differences that accompany age-related cognitive slowing remain fundamentally unknown. We sought to isolate the origin of age-related BOLD changes by comparing blood-flow and oxygen-metabolic constituents of the BOLD response using dual-echo arterial spin labeling during visual stimulation and CO$_2$ ingestion. We hypothesized, and our results confirmed, that age-related changes in the ratio of fractional cerebral blood flow to fractional cerebral metabolic rate of oxygen consumption ($\Delta$CBF/$\Delta$CMRO$_2$) lead to the BOLD changes that are observed in older adults. $\Delta$CBF/$\Delta$CMRO$_2$ was also significantly related to performance, suggesting that age-related cognitive slowing results from neural cell assemblies that operate less efficiently, requiring greater oxygen metabolism that is not matched by blood-flow changes relative to younger adults. Age-related changes in $\Delta$CBF/$\Delta$CMRO$_2$ are sufficient to explain variations in BOLD responding and performance cited throughout the literature, assuming no bias based on physiological baseline CMRO$_2$.

Keywords: aging, BOLD, CBF, CMRO$_2$, dual-echo ASL, hypercapnia, neural efficiency, processing efficiency

Introduction

Observations of age-associated performance declines have led to hypotheses suggesting that these declines are governed by reductions in fundamental resources (Hasher and Zacks 1988; Salthouse 1996). In particular, processing efficiency deficits have been implicated in slower reaction times (RTs) observed in older individuals across a variety of paradigms, ranging from visual tasks (Welford 1981; Speranza et al. 2001) to working memory tasks (Anders et al. 1972; Craik and Jennings 1992; Rypma et al. 2005).

Attempts to find indices of age-related resource decline have extended to age-group comparisons of blood-oxygen-level-dependent (BOLD) activity, as measured by the hemodynamic response function (HRF, the function that describes relationships between neural and vascular activity over time; Gössl et al. 2001) using functional magnetic resonance imaging (fMRI). Age-related increases and decreases in BOLD activity (Grady 2002; Hedden and Gabrieli 2004; Rajah and D’Esposito 2005; Nyberg and Bäckman 2011) are often interpreted as aging effects upon neural activity and form the basis for current neurocognitive aging hypotheses (Cabeza 2002; Park et al. 2002; Reuter-Lorenz and Cappell 2008). Neural activity, however, is but one among a set of factors from which the BOLD response arises (Hoge et al. 1999a) that could change with age and lead to age-related BOLD differences (Moeller et al. 1996; Davis et al. 1998; Hoge et al. 1999a; Rypma and D’Esposito 2000, 2001; D’Esposito et al. 2003; Iadecola 2004; Rypma et al. 2005; Leontiev et al. 2007; Restom et al. 2007; Ances et al. 2009; Motes et al. 2010). The HRF depends upon the integrity of numerous structures and physiological processes that change with age (such as cerebral blood flow [CBF]; D’Esposito et al. 2003; Buxton et al. 2004) and that remain poorly understood. For instance, age-related changes in cerebral vasculature have been observed that could potentially adversely affect accurate measurement of neural activity with BOLD signal (D’Esposito et al. 1999; D’Esposito et al. 2003) and its relationship to behavior.

The BOLD signal, in part, arises when synaptic activity trigger astrocytic control of vasodilating prostaglandins (Rossi 2006; Takano and Tsuchiya 2006) that mediate CBF, in response to cerebral metabolic rate of oxygen (CMRO$_2$) changes associated with neural activity (Davis et al. 1998; Hoge et al. 1999a, b; Hyder et al. 2001, 2002; Leontiev and Buxton 2007; Fig. 1; see Cauli and Hamel 2010 for review). Thus, cerebral activity leads to fractional increases in cerebral perfusion of oxygenated blood (ACBF) that exceed fractional increases in oxygen metabolic rate (ACMRO$_2$) for active neurons, leading to increases in the T2* magnetic resonance (MR) signal known as the BOLD response (Ogawa and Lee 1990; Ogawa et al. 1992). Because $\Delta$CBF and $\Delta$CMRO$_2$ vary independently, the $\Delta$CBF/$\Delta$CMRO$_2$ ratio can differ substantially across cortex and influence regional variation in the BOLD response. Few studies have extended this knowledge to hypotheses of age-related variation in BOLD activity and behavior (Mohtashib et al. 2012). We hypothesized that age-related changes in the performance are related to changes in the $\Delta$CBF/$\Delta$CMRO$_2$ ratio. Our study is the first to focus on these basic relationships within visual cortex, minimizing the complexities of strategic effects on functional activity.

Taken separately, $\Delta$CBF and $\Delta$CMRO$_2$ might partially account for the wide range of age-related fMRI results reported in the literature. However, because age-related changes in CBF and CMRO$_2$ are heterogeneous across cortex and tasks, age differences in the BOLD response could be differentially affected by fluctuations in one or both of these factors (Buxton et al. 2004). Taken together, the ratio of $\Delta$CBF to $\Delta$CMRO$_2$ is relatively stable (Leontiev and Buxton 2007) and allows for the heterogeneity of changes in both factors and therefore can potentially account for the region- and task-specific nature of BOLD age differences, and their relationships to performance, that have been documented across cortex. Therefore, we took the novel step of evaluating whether age-related $\Delta$CBF/$\Delta$CMRO$_2$ differences, and differences in components of this
ratio, lead to age-related differences in the BOLD response and behavior. Note that whereas \( \Delta \text{CBF/CMRO}_2 \) is a key component of the BOLD response, \( \Delta \text{CBF/CMRO}_2 \) and BOLD are not equivalent due to factors such as \( M \) (a calibration factor; Davis et al. 1998) that can vary across cortex, participants, and time (cf. Leontiev and Buxton 2007). It has been demonstrated to a certainty that, in intact systems (i.e. in healthy, young participants), increases in \( \Delta \text{CBF/CMRO}_2 \) lead to increases in BOLD (cf. Fig. 1; e.g. Hoge et al. 1999a; Stefanovic et al. 2004; Leontiev and Buxton 2007; Leontiev et al. 2007). Our hypothesis was that the relationship between \( \Delta \text{CBF} \) and \( \Delta \text{CMRO}_2 \) is fundamentally altered by the process of aging such that the BOLD response might differentially index neural activity in older compared with younger individuals.

To empirically evaluate the role of the \( \Delta \text{CBF/CMRO}_2 \) ratio in age-related BOLD differences and its relationship to performance, we utilized calibrated fMRI technology to assess visual cortex response to flickering annuli (Welford 1981; Hoge et al. 1999a, b; Pasley et al. 2007; Ances et al. 2009). In the “fixation” condition, BOLD activity generated in response to flickering annulus was compared with that generated during presentation of a fixation cross on a gray background. In the “parafeveal stimulation” condition, a competing stimulus known to inhibit visual cortex was presented prior to the stimulating annulus. During stimulus presentation, participants were required to press response buttons when they noticed a change in the luminance of a central fixation cross (see Fig. 2B; Pasley et al. 2007).

To determine individual subjects’ theoretical maximum BOLD response in the absence of deoxyhemoglobin (\( M \); Davis et al. 1998; Hoge et al. 1999a), we performed a separate hypercapnia scan during which subjects ingested room air and carbon dioxide (\( \text{CO}_2 \)) during 2 sequential time blocks. Hypercapnia induces vasodilation, an increase in venous oxygenation, and a washout of deoxyhemoglobin, which allows for the derivation of \( M \). We were then able to calculate \( \Delta \text{CMRO}_2 \) and \( \Delta \text{CBF/CMRO}_2 \) (Leontiev and Buxton 2007; Leontiev et al. 2007) from resting and parafeveal stimulation conditions for each individual. Some results have suggested that the CBF and BOLD (echo 1 and echo 2, responses, respectively) in calibrated fMRI do not have the same signal-to-noise ratio (Wong et al. 2000), potentially affecting the calculation of \( \Delta \text{CMRO}_2 \). Collection of hypercapnic (Davis et al. 1998) or hyperoxic (Chiarelli et al. 2007) data in tandem with calibrated fMRI ameliorates this concern to the extent that it allows for scaling of the BOLD response to account for differences in the signal-to-noise ratio. Previous work has shown the components of calibrated fMRI to be complementary to one another in elucidating the relationship between vasculature, neural activation, and the BOLD response (see Brown et al. 2007).

Our results showed that age-related increases in \( \Delta \text{CMRO}_2 \) led to significant age decrements in the \( \Delta \text{CBF/CMRO}_2 \) ratio and thus a lower BOLD signal for older adults. These results support the hypothesis that age-related BOLD changes are mediated by changes in \( \Delta \text{CBF/CMRO}_2 \). Further, a significant relationship between changes in \( \Delta \text{CBF/CMRO}_2 \) and performance suggests that age-related \( \Delta \text{CBF/CMRO}_2 \) changes observed at the physiological level play a central role in age-related processing efficiency reductions observed at the behavioral level.

Materials and Methods

Participants

Twenty-two healthy younger (age: 23–33, mean age: 28.2 years; \( n = 11 \), 6 females) and older (age: 53–72, mean age: 60.5 years; \( n = 11 \), 6 females) individuals participated. Recruits came from the web, from advertisements in circulars and flyers distributed throughout the Dallas-Fort Worth Metroplex, and from the University of Texas at Dallas (UTD) and the University of Texas Southwestern Medical Center (UTSWMC) campuses. All advertisements were approved by the UTSWMC and UTD Institutional Review Boards (IRBs).

No participants were taking blood pressure medications, diuretics, or psychotropic/psychoactive medications, and none reported any history of heart, lung, neurological, or psychiatric difficulties. All participants were screened and deemed safe within the 3 Tesla (3T) MR scanning environment. Participants were also cognitively intact based on assessments during the telephone prescreening (de Jager et al. 2003) and in person on the day of the scans (Fröstig et al. 1975). Participants were compensated at the rate of $100 for their time (~$50 per hour, including time to consent using a form approved by both the UTSWMC and UTD IRBs).

Visual Stimuli

Flashing sinusoidal grating annuli were presented to activate or depress activity within cortical regions of interest (ROIs), which were the visual areas corresponding to peripheral visual field (Pasley et al. 2007). Three types of stimuli were presented: Parafocal, or negative response, stimulus (1.7–3.3° eccentricity, drift temporal frequency = 6 Hz, spatial frequency = 1 cycle per degree, 80% contrast); peripheral, or positive response, stimulus (6.8–9.9° eccentricity, drift temporal frequency = 6 Hz, spatial frequency = 1 cycle per degree, 25% contrast); and combined stimuli (parafocal stimulus onset followed by peripheral stimulus onset after a 20 s delay, maintaining both images in view simultaneously after positive response stimulus onset). A fixation cross was presented in the center of the screen throughout the visual task. It changed in luminance every 3–7 s, with the mean time between luminance changes equal to 5 s. Stimuli were presented in 3 runs, with 2 instances of each stimulus block per run, randomized in order within each run, and with 30 s fixation periods spaced temporally between blocks, for a total of 6 instances of each stimulus block concatenated across runs. Each run lasted a total of 600 s (i.e. 10 min).
A 2-way nonrebreathing valve/mouthpiece combination (Hans Rudolph, 2600 series, Shawnee, KS, United States of America) was used to deliver a 5% CO2 solution (balanced with 21% O2 and 74% N2; contained in a Douglas bag) for hypercapnic induction. A capnograph device (Capnogard, Model 1265, Novametrix Medical Systems, CT, United States of America) was used to monitor end-tidal CO2, and a pulse oximeter (MEDRAD, Pittsburgh, PA, United States of America) was used to monitor breathing rate, heart rate, and arterial oxygenation saturation. Values from both devices were recorded on a Lenovo tablet PC running the Microsoft Vista operating system and using HyperTerminal (Private Edition, Version 6.3, by Hilgraeve, Monroe, MI, United States of America) for data collection.

**Hypercapnia Materials**

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**Apparatus and Scanning Parameters**

All imaging was conducted at the UTSWMC Advanced Imaging Research Center on a 3T MRI system (Philips Medical Systems, Best, The Netherlands). High-resolution anatomical data were acquired using a T1-weighted magnetization-prepared rapid acquisition of gradient echo (MPRAGE) pulse sequence (Brant-Zawadzki et al. 1992). Calibrated fMRI functionally incorporated both arterial spin labeling (ASL; echo 1, dependent on CBF) and BOLD (echo 2, dependent on both CBF and CMRO2). A pseudo-continuous ASL sequence (Garcia et al. 2005) was used to acquire the calibrated fMRI data (visual, hypercapnia) with echo times at TE1 = 11 ms and TE2 = 30 ms (Flip angle = 90°, 16 slices, 5 mm thick, orientation transverse, slice around calcarine sulcus linearly from bottom to top, TR = 4 s, 150 volumes). Global baseline CBF was measured in sagittal sinus (phase contrast voxel size 0.45x0.45x5 mm³, maximum velocity 80 cm/s, duration 30 s; note that hypercapnia-induced CBF increases are comparable for sagittal sinus and feeding arteries, which perfuse the entire brain; Aslan et al. 2010) using phase contrast scans that were run immediately prior to and immediately following the hypercapnia sequence, representing normocapnic and hypercapnic conditions, respectively. Phase contrast MRI data were used to normalize ASL signals as described previously (Aslan et al. 2010). Scans were acquired in the following sequence: High-resolution anatomical MPRAGE, 3 runs of the visual task, normocapnic phase contrast, hypercapnia, and hypercapnic phase contrast (Fig. 2C).

**Procedure**

The high-resolution anatomical MPRAGE was acquired first, followed by 3 runs of the visual task. Participants were asked to ignore the flashing annuli but to quickly press and release both buttons (incorporated into hand-held button boxes) every time they noticed the fixation cross changing in luminance—that is, appearing lighter or darker. This task served to maintain the participants’ focus on the center of the screen so that positive response cortical areas could be located functionally using calibrated fMRI.

At the conclusion of the visual stimulus runs, the patient table was withdrawn from the bore of the scanner and the mouthpiece for the hypercapnia portion of the experiment was placed into the participant’s mouth. The participant wiped the exterior of his or her nose with a moist towelette, the nose clip was placed on the participant’s nose, and it was verified that the participant could breathe comfortably and that the nonrebreathing valve was working properly. Button boxes were removed and a pulse oximeter finger monitor was clipped onto a finger of the participant’s left hand. An emergency squeeze bulb was positioned near the right hand such that the participant could easily squeeze it without having to move or look for the...
bulb. The patient table was returned to the scanner bore and the hypercapnia experiment procedure was executed, flanked by 2 4-min phase contrast scans (normocapnic and hypercapnic, respectively). A researcher stayed inside the magnet room throughout the phase contrast and hypercapnia portions of the experiment to manually switch the valve to control the breathing of air (either room air or CO₂ bag). At the termination of the final scan, the bag valve was returned to room air, the patient table was withdrawn from the bore of the scanner, and the hypercapnia equipment was removed from the participant.

**Data Pre-Processing**

Data were taken from Philips PAR/REC format and transformed into SPM (Statistical Parametric Mapping, Wellcome Department of Imaging Neuroscience, University College London, London, United Kingdom) Analyze format using the GUI for R2A Rec-to-Analyze Converter (Hermans and Neggers, GUI for R2A, version 2.1 released March 2006, modified February 2008, Helmholz Institute, Utrecht University, Dept of Brain Research, University Medical Center, Utrecht, Netherlands). Files were then spatially pre-processed in SPM using the Realign-Estimate-Reslice algorithm for each echo and run. In-house MATLAB® (Version 7.4, Mathworks, Natick, MA, United States of America) code separated Echo 1 and Echo 2 into separate Rec files for each echo and run, and the new Rec files were then read into AFNI (Analysis of Functional Neuroimages; Cox 1996) where the data were registered to the individual’s MPRAGE space and aligned to the first brick of the first run of Echo 2 (dummy scans prevented the need to discard the first few volumes). Parameters were saved and applied to all runs for both Echo 2 and Echo 1. Labeled images were subtracted from control images in Echo 1 to obtain CBF weighted images. Echo 2 data were subjected to a low pass temporal Fourier filter (0.05) and were then spatially smoothed (FWHM = 8). Noise was cleared from outside the head. A mask was then generated to restrict analyses to areas representing visual cortex—specifically, we utilized a morphometric approximation of Brodmann Areas (BAs; Brodmann 2006/1909) 17, 18, and 19. BAs were defined by the demarcation of landmarks in each individual’s native space using Caret (Van Essen et al. 2001); landmarks included those automatically generated by Caret prior to flattening (Medial Wall Dorsal, Medial Wall Ventral, and Calcarine Fissure), those recommended by Caret after flattening (the central sulcus, the Sylvian fissure, and the anterior half of the superior temporal gyrus; Van Essen, 2005), and those found by our laboratory to be consistently identifiable and accurate in deriving cleaner, more accurate BA demarcation across participants, also after flattening (inferior rostral sulcus, Pars Triangularis, Pars Orbitalis, inferior frontal sulcus, and superior temporal sulcus). Native-space landmarks were spherically registered to a Colin-brain template incorporating all BAs, and these then transformed into each individual’s native space (cf. Fig. 2A). Functional activation in response to the visual stimuli was used to narrow ROI selection as described below.

**Data Analyses, Visual Data**

Echo 1 and Echo 2 data, reflecting CBF and BOLD, respectively, were analyzed using 3DDeconvolve to conduct a regression analysis in AFNI (Cox 1996). Beta values, along with their associated P values, were generated for each individual for each stimulus condition for each echo. For further analysis, ROI selection was based on functional activation overlap between CBF and BOLD but was restricted to the morphometrically defined visual cortical areas BAs 17, 18, and 19 (see above). In light of evidence suggesting that the dependent measures of interest might be less prone to bias if blood flow is taken into account and ROIs are sufficiently large (Leontiev et al. 2007), we required overlap of CBF and BOLD activation but selected a generous thresholding measure (Poline et al. 2006) such that t values of 1.00 were adequate for positive and negative response conditions, and the combined condition had no statistical thresholding. Thus, in order for a voxel to be retained within the ROI, it had to: 1) react positively to the peripheral stimulus for both CBF and BOLD, 2) react negatively to the parafoveal stimulus for both CBF and BOLD, 3) react positively to the combined stimulus for both CBF and BOLD, and 4) be contained within the morphometrically defined BAs 17, 18, and 19 (cf. Fig. 2D). ROIs for individuals ranged from 6 to 269 voxels (younger: 18–269 voxels, mean = 128.7, standard deviation [SD] = 81.7; older: 6–136 voxels, mean = 65.4, SD = 41.3). Concerns regarding biases introduced by ROI size differences between the groups are obviated by our results, which indicate a pattern opposite to previous observations that smaller functional ROIs tend to be associated with larger ΔCBF/ΔCMRO₂ ratios (Leontiev et al. 2007), and thus typically larger BOLD responses. All results reported here were averaged over the ROI for the stimulus condition of interest unless specifically stated as being calculated over the whole brain.

Beta values from the regression analysis were adjusted to reflect percent signal change from fixation (for echoes 1 and 2, respectively) and were then masked by the ROI selection that included the overlap area between CBF and BOLD activations to obtain mean values per stimulus condition for younger and older groups. Additional statistical analyses were conducted using SAS® software program (Version 9.1, SAS Institute, Cary, NC, United States of America) outside of brain space.

**Data Analyses, Hypercapnia Data**

Data in Echo 1 were used to obtain CBF weighted images and data in Echo 2 represented the BOLD images, as described above. The ROI masks defined in the visual fMRI runs were applied to the hypercapnia data to obtain time courses of CBF and BOLD signals. The CBF and BOLD percent signal changes were calculated comparing the average hypercapnia signal (volumes 91–150) to the average normocapnia signal (volumes 1–60).

Continuing to use in-house Matlab code, we used the Davis et al. (1998) and Hoge et al. (1999a) model to estimate M (a value necessary for the calculation of CMRO₂; Davis et al., 1998) from calibrated fMRI hypercapnia data: M = BOLD percentage/(1 – (1 + CBF percentage) exp(α – β)), with the assumption that CMRO₂ and neural activity are not significantly affected by hypercapnic conditions (Hoge et al. 1999b). We assumed α equals 0.38 (Grubb et al. 1974) and β equals 1.33 (Lu and van Zijl 2005); Hoge et al. (1999a) demonstrated that although estimates of α and β affect asymptotic extrapolations of M, these values do not significantly impact results in the range of human imaging studies, and so the utilization of estimates for α and β was deemed adequate. M was calculated both over the ROI only and over the entire brain. There is some evidence that age-related differences in M can lead to differences in the BOLD response (Ances et al. 2009, but see Hoge et al. 1999a). To be thorough, we investigated this possibility and did not find significant differences in M values between younger (mean M = 0.10, SD = 0.06) and older (mean M = 0.12, SD = 0.05) groups, t(20) = 0.92, P = 0.37, nonsignificant, over the functionally derived ROI; comparing M values averaged over the entire brain between the younger and older groups was similarly nonsignificant.

Finally, using M from the visually derived ROI, we were able to calculate ΔCMRO₂ and ΔCBF/ΔCMRO₂ for each individual, both in terms of change from fixation and from parafoveal stimulation. (One younger participant had a ΔCBF/ΔCMRO₂ ratio exceeding 2.5 SD greater than the remainder of the group. In order to present a clearer picture of the mean group values, this participant’s ΔCBF/ΔCMRO₂ ratio data are not included in the analyses presented in the Results, although the inclusion of this participant’s data did not alter the significance of the results.) Similar ratios were calculated for BOLD and CBF data. The Davis et al. (1998) and Hoge et al. (1999a) model was also the basis for these calculations. Because there were no significant differences between groups in their M values, we repeated our analyses using the mean M for each group (older and younger) and a single mean M value that collapsed across both groups. ΔCBF/ΔCMRO₂ values were still significantly different between the groups. Values reported in the paper use individual M values (cf. Chiarelli et al. 2007). ΔCBF/ΔCMRO₂ was not normally distributed across the sample from either fixation, W = 0.74, P = 0.0001, or from parafoveal stimulation, W = 0.81, P = 0.0008. Kruskal–Wallis tests were used to assess group differences under such conditions of nonnormality.
Results
A priori hypotheses of agerelated changes in ΔCBF/ΔCMRO₂ ratios and their relationships to behavior were tested using planned comparisons (cf. Keppel and Wickens 2004). For additional tests we calculated, using false discovery rate (FDR) theory (Benjamini and Hochberg 2000), that we would expect 0.85, or roughly between 0 and 1, of these tests to falsely reject the null hypothesis (0.05*17 = 0.85). For these tests, we rejected the null hypothesis at P < 0.05, FDR < 0.05. Thus, it is probable that our tests correctly rejected our null hypotheses, particularly given that the results portray a consistent picture. For the sake of clarity, the FDR is reported in addition to P when P < 0.05 and FDR < 0.10.

Behavioral Results
Younger participants (mean RT = 553.93 s, standard error of the mean [SEM] = 31.21) were significantly faster in responding to the fixation-cross luminance change than older participants (mean RT = 677.66 s, SEM = 34.88), t(20) = -2.86, P < 0.01. Younger participants were also more accurate (mean proportion correct = 0.83, SEM = 0.02) than their older counterparts (mean proportion correct = 0.69, SEM = 0.05), t(13.5) = 2.61, P = 0.02. There was no evidence of a speed-accuracy trade-off, as RT was negatively associated with proportion correct, Spearman’s ρ = -0.67, P = 0.0007.

Visual Stimulation: BOLD
For the visual experiment (see Fig. 2B and C), we first sought to test the a priori hypothesis of an age-related difference in the BOLD response, as the etiology of this difference was the topic under study. A general linear model (GLM) incorporating group (younger and older), stimulus conditions of interest (from fixation: Parafoveal, peripheral, and combined; from parafoveal stimulation: combined), and a group by stimulus condition interaction, was significant for determining percent signal change in the BOLD response, F(7,80) = 68.93, P < 0.0001, R² = 0.86. As seen in Figure 3A, in addition to differences based on stimulus type, the younger group had a greater BOLD response than the older group, F(1,80) = 8.18, P = 0.005.

ACBF/ΔCMRO₂ Ratio
To test the a priori hypothesis that ΔCBF/ΔCMRO₂ differences could mediate age-related changes in the BOLD response, ΔCBF/ΔCMRO₂ was calculated for each individual from both resting and parafoveal stimulation conditions (see Fig. 4A). ΔCBF/ΔCMRO₂ was significantly greater for the younger group than the older group, both from fixation (younger: mean = 2.40, SEM = 0.24; older: mean = 1.88, SEM = 0.06), Kruskal–Wallis χ²(1) = 4.17, P = 0.04, and from parafoveal stimulation (younger: mean = 2.00, SEM = 0.12; older: mean = 1.69, SEM = 0.05), χ²(1) = 4.46, P = 0.03 (Fig. 4B). These results support the hypothesis that age-related BOLD signal changes are the consequence of differences in the ratio of cerebral perfusion to oxygen metabolic rate (i.e. ΔCBF/ΔCMRO₂) between the younger and older groups, and these age-related differences occur regardless of baseline stimulation condition (i.e. fixation or parafoveal). Some studies (e.g. Leontiev et al. 2007) have shown a pattern in which smaller functional ROIs tend to be accompanied by greater ΔCBF/ΔCMRO₂ ratios. Greater amounts of noise in the data could restrict the ROI size and inflate ΔCBF/ΔCMRO₂ ratio estimates. If our results were attributable to this ROI size bias, we would expect larger ΔCBF/ΔCMRO₂ ratios for the older group in comparison to the younger group, and yet we observed the opposite. Moreover, a correlation between percent signal change (CBF and BOLD) and ROI size was not significant (P = 0.34 and 0.15, respectively), suggesting that our results do not reflect ROI size differences.

Components of the ΔCBF/ΔCMRO₂ Ratio
Significant differences in the CBF response would indicate that age-related differences in the BOLD response are at least partially due to blood flow changes that occur with age-related perfusion (i.e. blood flow) changes. As with the BOLD data, we utilized a GLM incorporating group, stimulus conditions of interest (from fixation: Negative, positive, and combined; from parafoveal stimulation: combined), and a group by stimulus condition interaction. The omnibus F statistic indicated that this model was significant for determining percent signal change in the CBF response, F(7,80) = 66.42, P < 0.0001, R² = 0.85. As seen in Figure 3B, the CBF difference

Figure 3. CBF and BOLD responses to visual stimulation. (A) BOLD percent signal change (mean ± SEM) for younger and older groups based on visual stimulation shown by stimulus condition. Significant differences between groups are indicated by asterisks: *P < 0.05, **P < 0.004. (B) CBF percent signal change (mean ± SEM) for younger and older groups based on visual stimulation shown by stimulus condition. (Comparison of the combined condition to parafoveal stimulation, shown in the rightmost bars of Fig. 3A–B, is aggregated from the parafoveal and combined stimulus conditions for both BOLD and CBF—that is, the effects are additive. Although this is not an independent measurement, it is shown here for illustrative purposes).
between younger and older groups was not significant, $F_{1,80} = 0.32$, $P = 0.57$, indicating that age-related differences in ΔCBF/ΔCMRO2 were not caused by differences in the CBF response. Additionally, there were minimal differences in “absolute” (i.e. change from fixation) CBF in the older compared with the younger group, both from fixation, $\chi^2(1) = 0.24$, $P = 0.62$ (see Fig. 5A), and parafoveal stimulation, $\chi^2(1) = 0.05$, $P = 0.82$ (see Fig. 5B).

We also sought to determine if there were age-related differences in the ΔCMRO2 component of ΔCBF/ΔCMRO2. We observed age-related ΔCMRO2 differences in “absolute” measures (i.e. change from fixation; Fig. 5A) when measured from parafoveal stimulation, $\chi^2(1) = 4.28$, $P < 0.04$ (Fig. 5B; FDR < 0.09). Additionally, we observed greater “incremental” (i.e. change from respective baseline stimulation condition—that is, either fixation or parafoveal stimulation) evoked ΔCMRO2 from parafoveal stimulation (least squared mean [LSM] = 0.30, SEM = 0.02) than from fixation (LSM = 0.16, SEM = 0.02), $t(21) = 11.17$, $P < 0.0001$; this effect was also found for BOLD and ΔCBF, $P < 0.0001$. However, only the BOLD response showed greater incremental evoked responses for the younger (mean = 0.0094, SEM = 0.0007) than the older group (mean = 0.0073, SEM = 0.0007), $t(20) = 2.09$, $P < 0.05$ (FDR = 0.09), indicating that age-related differences in ΔCMRO2 are not attributable to baseline stimulation condition differences. In sum, comparing differences between younger and older participants on the constituents of the BOLD response revealed minimal differences in ΔCBF but significant age-related differences in ΔCMRO2 (Figs 4C, 5A, B). This change in ΔCMRO2 affected significant change in ΔCBF/ΔCMRO2.

**ΔCBF/ΔCMRO2: Relationships to Behavior**

The behavioral significance of age-related ΔCBF/ΔCMRO2 differences could be demonstrated if we observed relationships between this physiologic factor and individual subject performance. A Spearman’s $\rho$ test of association across younger and older groups confirmed our a priori hypothesis that RT was significantly negatively associated with ΔCBF/ΔCMRO2 from fixation, $\rho = -0.49$, $P = 0.02$ (and from parafoveal stimulation, $\rho = -0.43$, $P = 0.05$). Figure 4C illustrates this relationship when broken out by group (younger or older) and RT (slower or faster, as determined by within-group median split). Likewise, a Spearman’s $\rho$ test of association confirmed our a priori hypothesis that proportion correct was positively associated with ΔCBF/ΔCMRO2 from fixation, $\rho = 0.46$, $P < 0.04$. Neither RT nor proportion correct was associated with the ΔCBF or ΔCMRO2 components separately. These results support the hypothesis that age-related behavioral indices—that is, slowing of RTs and reduced proportion
correct—are significantly associated with age-related reductions in $\Delta$CBF/$\Delta$CMRO$_2$.

**Discussion**

Because the ASL signal is dependent on CBF and the BOLD signal is dependent on both CBF and CMRO$_2$, use of dual-echo ASL (i.e. calibrated fMRI) allowed separation of vascular and neural factors and permitted resolution of age-related BOLD signal ambiguities. Our goal was to evaluate whether $\Delta$CBF/$\Delta$CMRO$_2$ mediates age-related differences in the BOLD response. The BOLD response was significantly diminished for the older compared with the younger group in visual cortex, whereas $\Delta$CBF responses were equivalent between groups. $\Delta$CMRO$_2$ was greater in the older group, leading to our observations of reduced $\Delta$CBF/$\Delta$CMRO$_2$ for the older group and a reduced BOLD signal to visual flashing annuli, regardless of baseline stimulation condition. Our results implicate a mechanism by which age-related changes in the BOLD response to neural activity arise. They suggest that $\Delta$CMRO$_2$ increases, not accommodated by $\Delta$CBF increases, lead to reduced fMRI signal in older adults. Further, performance reductions (greater RT and reduced proportion correct in older compared with younger) were significantly associated with reduced $\Delta$CBF/$\Delta$CMRO$_2$, suggesting that alterations to $\Delta$CBF/$\Delta$CMRO$_2$ affected the processes by which the visual stimuli were perceived.

Conclusions regarding age-equivalent $\Delta$CBF and age-differential $\Delta$CMRO$_2$ rest on assumptions of linearity between CBF and CMRO$_2$ and age-equivalent baselines. Several studies suggest linear relationships between CBF and CMRO$_2$ (e.g. Hoge et al. 1999a, b; Stefanovic et al. 2004) but others do not (e.g. Lin et al. 2010). Age-related increases in $\Delta$CMRO$_2$ have been observed in some studies assessing different physiological baseline CMRO$_2$ levels between younger and older groups (cf. Hyder et al. 2002). Such baseline differences could influence measured changes in $\Delta$CMRO$_2$. For example, the elderly might have lower resting values of CMRO$_2$ as suggested by $^{13}$C MR spectroscopy (Gjedde 1997; Boumezbeur et al. 2010) and positron emission tomography (e.g. Kety 1956; Marchal et al. 1992). In such a mechanism, the greater CMRO$_2$ change that we observed in the elderly compared with young could reflect activity increases that result in age-equivalent absolute neural activity under stimulation conditions as opposed to a larger neural response (cf. Hyder et al. 2002). Age-differential CMRO$_2$ baseline levels (cf. Hyder et al. 2002) could also influence blood flow response. In such a mechanism, as CMRO$_2$ exceeded resting levels, the fractional increase in CBF needed to supply the increment in oxygen consumption would increase asymptotically as venous pressure of oxygen (pO$_2$) approached arterial values resulting in increasing $\Delta$CBF/$\Delta$CMRO$_2$, as a function of absolute CMRO$_2$ (cf. Buxton and Frank 1997; Lin et al. 2010). In such a mechanism, the increased $\Delta$CBF/$\Delta$CMRO$_2$ we observed in younger subjects would reflect their higher resting physiological CMRO$_2$ rather than an alteration in vascular response.

Other research suggests, however, that baseline CMRO$_2$ actually increases with age after accounting for reductions in brain parenchyma volume (Lu et al. 2011). Thus, if age-related CMRO$_2$ baseline changes were driving the age-related differences in $\Delta$CMRO$_2$ during activation, greater $\Delta$CMRO$_2$ for younger than for older adults would be expected—the opposite of the result we obtained. Additionally, we measured $\Delta$CMRO$_2$ from 2 baseline stimulation conditions (i.e. fixation, parafoveal stimulation) to test just such a possibility. Our results showed an increase in $\Delta$CMRO$_2$ with aging that was greater from parafoveal stimulation than from fixation for both younger and older groups. Although this result does not conclusively demonstrate age-equivalent physiological baselines, it does indicate that CMRO$_2$ increments from different baseline conditions are proportional for both younger and older adults. This result does not support the hypothesis that age-differential $\Delta$CMRO$_2$ results from age-differential CMRO$_2$ baselines. Future research is certainly needed to clarify the nature of CBF/CMRO$_2$ linearity and the role of baseline CMRO$_2$ activity in assessing age-related BOLD signal change.

This study focused on activation-induced $\Delta$CMRO$_2$. Our interpretation of the results assumes that $\Delta$CMRO$_2$ reflects...
alterations in neural activity. There is some evidence that metabolic demand of evoked neural activity might be met by glucose metabolism (e.g. Raichle and Mintun 2006). We note that the rate of glucose consumption (CMRglu) is usually coupled to oxygen metabolic rate. Therefore, we expect that the CMRglu changes (ΔCMRglu) in our experiment would be similar to the measured ΔCMRO2. Furthermore, the time that ΔCMRO2 and ΔCMRglu are most likely to be decoupled is during the first several seconds after stimulation onset (prior to CBF response). Because we used a block design with stimulus durations of at least 20 s, we consider ΔCMRO2 measurement as an accurate reflection of ΔCMRglu and neural activity (cf. Lin et al. 2010).

Variance between studies comparing the cerebrovascular characteristics of younger and older adults (Ross et al. 1997; Taoka et al. 1998; D’Esposito et al. 1999; Huettel et al. 2001; Restom et al. 2007; Ances et al. 2009; Lu et al. 2011) suggest that age differences in the BOLD response arise from a complex and variable relationship between ΔCBF and ΔCMRO2 such that ΔCBF increases lead to BOLD amplitude increases whereas ΔCMRO2 increases lead to BOLD amplitude decreases (Buxton et al. 2004). The present results, showing age-related ΔCBF/ΔCMRO2 alterations in primary visual regions, suggest that prior results might be more consistent than previously thought.

In regions where age-related changes in ΔCBF and ΔCMRO2 occur (Heo et al. 2010; Chen et al. 2011; Lu et al. 2011), the ΔCBF/ΔCMRO2 ratio could differ substantially between younger and older groups leading to apparent age differences, or changes in one factor could offset changes in the other factor leading to apparent age-equivalence. For example, age-equivalent BOLD signal has been observed in medial temporal lobe in numerous studies (e.g. Morcom et al. 2003; Persson et al. 2011), even in the presence of ΔCBF increases (Restom et al. 2007). Our results support the conclusion that these results are more a reflection of age-related increases in ΔCMRO2 minimized by the effects of increased ΔCBF, than age-equivalence in neural activity per se (cf. Restom et al. 2007). In contrast to results in medial temporal lobe, our results show that the visual-cortex ΔCBF/ΔCMRO2 ratio diminishes with age due to age-differential increases in ΔCMRO2, with age-equivalence in ΔCBF leading to decreases in BOLD responding in older compared with younger groups (cf. Ross et al. 1997). Such cross-cortex variability (Rypma and D’Esposito 2001; Rypma et al. 2006; Rypma and Prabhakaran 2009) has important implications for neurocognitive aging hypotheses based on BOLD signal because it suggests that regional BOLD age-differences, or age-equivalence, cannot be unambiguously interpreted in terms of neural activity.

Regional variation in ΔCBF/ΔCMRO2 might also mediate disease-related BOLD signal change. For example, in schizophrenia there is a significant shift in BOLD signal patterns across cortex compared with controls that is correlated with performance declines (Callcott et al. 2000; Winterer et al. 2006; Yoon et al. 2008). Winterer et al. (2006), for instance, observed that the prefrontal BOLD signal of schizophrenic patients was more diffuse, more variable, and less organized than that of healthy individuals, and those indices were related to slower RTs.

Likewise, cognitive training results in regional BOLD signal variation in healthy samples that might indicate experience-related shifts in ΔCBF/ΔCMRO2. Specifically, cross-cortical BOLD signal decreases over time with practice. This pattern suggests that task-specific knowledge might be focused across many brain regions for initial, effortful, task execution, and learning, but is then streamlined for skilled task execution (Petersen et al. 1998). Poldrack et al. (1998) observed BOLD signal decreases and increases across differing cortical regions with both skill learning and item-specific learning. Seger et al. (2000) also found learning-related shifts in BOLD signal patterns, from mainly right-hemisphere early in learning to bilateral BOLD signal later in learning. Thus, it might be that cortical variations in ΔCBF/ΔCMRO2 could change across cortical regions, reflecting intervening processes of age, disease, learning, and other influences.

Our results further suggest intriguing relationships between variation of ΔCBF, ΔCMRO2, and behavior. The associations we observed between age-related performance reductions and reductions in the ΔCBF/ΔCMRO2 ratio support the hypothesis that increased oxygen demand in the aging neural apparatus, not accommodated by vascular activity, is the basis for reduced functional efficiency at the neural level and reduced processing efficiency at the cognitive level (assuming no CMRO2-baseline induced bias). While further research is required, these results provide important clues to the link between the neurophysiologic changes, and the pervasive behavioral changes, that accompany human aging.

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