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Cortical Depth Dependence and Implications on the Neuronal Specificity of the Functional Apparent Diffusion Coefficient Contrast

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Abstract

Although the blood oxygenation level-dependent (BOLD) contrast is widely used in functional MRI (fMRI), its spatial specificity is compromised by the diversity of the participating vasculature, including large draining veins. Previous studies have shown that an alternative contrast mechanism based on functional changes of the apparent diffusion coefficient (ADC) can be sensitized to small vessels more closely tied to the sites of neural activity. Such an improved functional localization, however, has not yet been demonstrated at the cortical level in humans. Here, we investigate the cortical depth dependence and neuronal specificity of the functional ADC contrast in the human primary visual cortex by performing high-resolution BOLD and ADC imaging during visual stimulation at 4 T. Our results show that, by using optimal parameters, the functional ADC changes are significantly higher in the middle cortical layers, whereas the BOLD signal changes are higher at the cortical surface and vary much less significantly across the cortex. These results are in good agreement with previous studies performed in anesthetized cats at 9.4 T and demonstrate the improved spatial specificity of the functional ADC contrast as compared to the BOLD contrast.

Keywords

Apparent diffusion coefficient (ADC); Blood oxygenation level-dependent (BOLD); Functional MRI (fMRI); Cortical depth; Spatial specificity

Introduction

Since its inception, the blood oxygenation level-dependent (BOLD) contrast (Ogawa et al., 1993) has been extensively used in functional MRI (fMRI) because of its relatively high sensitivity. However, its spatial specificity is compromised by the diversity of the participating vasculature; for example, the contributing large draining veins are often distant from the sites of neural activity (Lai et al., 1993). A new contrast mechanism based on functional changes of the apparent diffusion coefficient (ADC) (Song et al., 2002) was previously proposed as an alternative to the BOLD contrast.

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Under mild diffusion-weighting (i.e., with b -factors ranging from 0 to ~ 200 s/mm²) sensitive to the intravoxel incoherent motion (IVIM) (Le Bihan et al., 1986), the ADC contrast predominantly originates from the vasculature. Previous studies have shown that the ADC activation temporally precedes the BOLD activation by about 1 s, with overlapping but also spatially distinct activated regions, suggesting that it mainly originates from capillaries and upstream arterial networks, as opposed to the BOLD contrast, which originates from capillaries and downstream venous networks (Gangstead et al., 2002; Song et al., 2004). Furthermore, by selecting an appropriate range of diffusion-weighting, the signal contributions from large arteries with a high blood velocity can be reduced. The ADC contrast can thus be tuned to be sensitive to small vessel networks (i.e., arterioles and capillaries), which are more closely tied to the sites of neural activity, thereby resulting in an improved functional localization (Song et al., 2004; Song et al., 2003).

More recently, high-resolution studies in anesthetized cats at 9.4 T have shown that when the venous blood contributions are minimized (i.e., at long echo times (TEs)), the functional ADC changes are mainly caused by an increase in arterial blood volume and show a better spatial localization to the middle cortical layer as compared the BOLD contrast (Jin et al., 2006). These results demonstrate the improved spatial specificity of the functional ADC contrast, because the middle cortical layer contains mostly microvessels (arterioles, capillaries, and venules), whereas the upper cortical layer contains larger arteries and veins. Such an improved functional localization, however, has not yet been demonstrated at the cortical level in humans. In the present work, we investigate the cortical depth dependence and neuronal specificity of the functional ADC contrast in the human primary visual cortex by performing high-resolution BOLD and ADC imaging during visual stimulation at 4 T.

Methods

MRI Experiments

Healthy adult volunteers, who provided written informed consent as approved by our Institutional Review Board, were studied on a General Electric Signa 4 T whole-body MRI scanner (GE Healthcare, Waukesha, WI) equipped with a 40 mT/m maximum amplitude and 150 T/m/s slew rate gradient system. Images were acquired using a whole-volume head coil and a four-channel visual array surface coil (Nova Medical, Inc., Wilmington, MA) specifically designed to achieve a high sensitivity in the occipital lobes. Five oblique slices covering the primary visual cortex and orthogonal to the calcarine fissure were acquired using a gradient-echo single-shot spiral-out imaging sequence with the following parameters: repetition time (TR) = 1 s, TE = 46 ms, flip angle = 60°, field-of-view = 9.6 cm, matrix size = 96×96 (zero-filled to 128×128), and slice thickness = 5 mm with no gap. High-resolution T_1 -weighted images were also acquired using a three-dimensional fast SPGR sequence with an isotropic resolution of 1×1×1 mm³ for anatomical reference. Automatic whole-volume high-order shimming was used to minimize the global B_0 inhomogeneity.

Simultaneous BOLD and ADC imaging was achieved by applying isotropic diffusion-weighting gradients with three b -factors of 2, 104, and 208 s/mm² cyclically interleaved within the same run (Song et al., 2002). A dynamic BOLD contrast was obtained by extracting the image volume time series acquired with $b = 2$ s/mm², whereas a dynamic ADC contrast was generated by fitting a monoexponential decay to the three image volume time series acquired at each b -factor. The lower bound for the b -factor range was set at 2 s/mm² to reduce the signal contributions from large arteries with a high blood velocity (> 50% signal reduction for vessels with a velocity > 2.3 cm/s), and thus sensitize the ADC contrast to small vessels (Song et al., 2004). The upper bound was selected to maximize the ADC contrast, based on our previous work (Song et al., 2002). In addition, isotropic diffusion-weighting gradients (Wong et al.,

1995) that have no cross-terms with the deoxyhemoglobin-induced background gradients were used to ensure that the measured ADC contrast is independent from the BOLD contrast.

The activation paradigm was a block design consisting of three 30-s “stimulation” periods interleaved within four 30-s “rest” periods, during which subjects passively viewed a rotating and flashing checkerboard or a black screen, respectively. The visual stimulus was delivered through MR-compatible video goggles (Resonance Technology, Inc., Northridge, CA), and subjects were instructed to fixate on a small cross displayed at the center of the visual field at all times. Following eight dummy scans to ensure that the MR signal reached a steady state, a total of 210 image volumes were acquired for each run (70 at each b -factor). Fifteen runs were acquired for each study to increase the signal-to-noise ratio (SNR), and three studies were performed to demonstrate the test-retest reliability.

Data Analysis

For each study, the image volume time series were first motion corrected, averaged across all runs, linearly detrended, and temporally realigned using spline interpolation to account for the interleaved slice acquisition. BOLD and ADC time courses were then derived as described above. A Student's t -test was performed pixel-by-pixel to detect significant differences in BOLD signal intensity or ADC value between rest and stimulation conditions, which were defined as the first 30-s rest period and the plateau of each stimulation period, respectively. The resulting t -score maps were then converted to Z-score maps, thresholded at $Z > 3$ (corresponding to a significance level of $P < 10^{-3}$ uncorrected for multiple comparisons) and with a cluster size of five voxels, and overlaid on the coregistered T_1 -weighted anatomical images to generate BOLD and ADC activation maps.

In addition, maps of the baseline ADC (ADC_{rest}), functional ADC change ($\Delta ADC/ADC_{rest} = (ADC_{stim} - ADC_{rest})/ADC_{rest}$), and BOLD signal change ($\Delta S/S_{rest} = (S_{stim} - S_{rest})/S_{rest}$) were also computed (with ADC_{rest} and S_{rest} defined as the mean over the first 30-s rest period, and ADC_{stim} and S_{stim} defined as the mean over the plateau of each stimulation period). The anatomical images were segmented into gray matter (GM), white matter (WM), and cerebrospinal fluid (CSF) masks, and the resulting WM/GM boundaries and GM/CSF boundaries (i.e., the cortical surface) overlaid on all maps. The contrast-to-noise ratio (CNR) of the ADC and BOLD activation was computed as the functional ADC or BOLD signal change (i.e., ΔADC or ΔS) divided by the standard deviation of the baseline ADC or BOLD signal during the first 30-s rest period, respectively.

To investigate the cortical depth dependence of the functional ADC and BOLD contrasts, ADC_{rest} , $\Delta ADC/ADC_{rest}$, and $\Delta S/S_{rest}$ profiles perpendicular to the cortical surface were computed as a function of the cortical depth (i.e., the distance from the cortical surface) on the upper and lower bank of the calcarine fissure, on the left and right hemisphere, and on multiple slices, and then averaged together. For these calculations, all maps were interpolated to 256×256 using bilinear interpolation, and the profiles computed up to a cortical depth of about 2 mm, which corresponds to the cortical thickness at the calcarine fissure (Zilles, 1990). Finally, the same analysis was repeated for each study, and the resulting profiles averaged across all studies. All data analysis was performed in Matlab (The MathWorks, Inc., Natick, MA).

Results and Discussion

Representative ADC and BOLD activation maps are shown in Figure 1. The ADC activation is relatively focal and predominantly localized within the GM mask (Fig. 1B), although there is also some activation within the WM mask near the WM/GM boundaries, most likely due to partial volume averaging in the slice direction, because a relatively large slice thickness (5 mm) as compared to the in-plane spatial resolution (1 mm) was used in this work to ensure a

sufficient SNR. In contrast, the BOLD activation at the same statistical threshold ($Z > 3$) extends throughout the entire visual cortex within all three GM, WM, and CSF masks (Fig. 1C), and still remains widespread and continuous across all three masks even when using a very high threshold ($Z > 7.5$) (Fig. 1D), because of signal contributions from vessels of different sizes, including large draining veins distant from the sites of neural activity (e.g., in the interhemispheric fissure). The average CNR of the ADC activation in the activated voxels (Fig. 1B) is 2.65, whereas the average CNR of the BOLD activation in the same region is 5.44.

The corresponding ADC_{rest} , $\Delta ADC/ADC_{rest}$, and $\Delta S/S_{rest}$ maps are shown in Figure 2, and their cortical depth dependence averaged within a single subject are shown in Figure 3A–C. As expected, the baseline ADC is the highest at the cortical surface (blue lines in Fig. 2A) because of partial volume effects with CSF, then decreases to a GM value of $(0.60 \pm 0.08) \times 10^{-3} \text{ mm}^2/\text{s}$ in the middle cortical layers, and finally increases to a WM value of $(0.75 \pm 0.08) \times 10^{-3} \text{ mm}^2/\text{s}$ when reaching the GM/WM boundary at a cortical depth of about 2 mm (Fig. 3A). The functional ADC change, on the other hand, is generally localized within the cortex (Fig. 2B), and is significantly higher in the middle cortical layers (Fig. 3B). Conversely, the BOLD signal change is higher at the cortical surface (Fig. 2C) because of signal contributions from large draining veins, and decreases continuously across the cortex (Fig. 3C). The average functional ADC change increases by a factor 5.1 from the cortical surface to its peak value at a cortical depth of about 1 mm, whereas the average BOLD signal change decreases by only 14% over the same distance. The ADC_{rest} , $\Delta ADC/ADC_{rest}$, and $\Delta S/S_{rest}$ profiles averaged across all three studies (Fig. 3D–F) are very similar to those obtained from a single subject (Fig. 3A–C). Despite slight variations in the magnitude of the functional ADC and BOLD signal changes across subjects, their cortical depth dependence remains virtually identical, thus demonstrating the consistency of the results.

Although the in-plane spatial resolution in our human studies (1 mm) is not as high as that used in previous animal studies (0.3 mm) (Jin et al., 2006) and is not sufficient to resolve individual cortical layers, and although the functional ADC change in our studies is an order of magnitude larger because of differences between species (awake humans vs. anesthetized cats), field strengths (4 T vs 9.4 T), and pulse sequences (gradient-echo vs. spin-echo), our results are nevertheless in good agreement in that they demonstrate that the functional ADC changes are better localized to the middle cortical layers as compared to the BOLD contrast. These results confirm that, for our choice of b -factors and imaging parameters, the signal contributions from large arteries and veins at the cortical surface are not significant and that the functional ADC contrast can be sensitized to small vessel networks more closely tied to the sites of neural activity, which is consistent with our previous studies based on the temporal characteristics of the ADC activation (Song et al., 2004).

In this work, we used isotropic diffusion-weighting gradients that have no cross-terms with the deoxyhemoglobin-induced background gradients to ensure that the measured ADC contrast is independent from the BOLD contrast. If diffusion-weighting gradients were applied along one direction, their coupling effects with the background gradients could lead to inaccuracies in the ADC measurements (Zhong et al. 1998; Does et al., 1999; Jin et al., 2008). However, we do not expect that the cortical depth dependence of the functional ADC contrast would be significantly different, as demonstrated by the previous animal studies, which used directional diffusion-weighting gradients (Jin et al., 2006).

In the present work, dynamic ADC imaging was performed by using repeated excitations with three interleaved b -factors, which limited the temporal resolution and statistical power, and potentially introduced inaccuracies in the ADC derivation resulting from signal changes due to subject motion, physiological noise, and/or system instabilities occurring between successive excitations. To address these limitations, we recently proposed a single-shot ADC

imaging technique (Song et al., 2007), in which a gradient-echo and two diffusion-weighted spin-echoes are acquired within a single excitation to generate an ADC map, thus resulting in a significant improvement in temporal resolution (by a factor 3), sensitivity (by a factor $\sqrt{3}$ (3) for the same scan time), and accuracy. It is anticipated that this novel technique, combined with parallel imaging methods such as SENSE, can lead to a much improved functional localization both spatially and temporally.

Conclusions

Our high-resolution BOLD and ADC fMRI studies performed in human subjects at 4 T during visual stimulation have shown that, by using optimal parameters, the functional ADC changes are better localized to the middle cortical layers as compared to the BOLD contrast, thus confirming previous results obtained in anesthetized cats at 9.4 T and demonstrating the improved spatial specificity of the functional ADC contrast. These results provide further evidence that this contrast mechanism can achieve a more accurate localization of neural activity in human fMRI studies.

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Fig. 1. T_1 -weighted anatomical image (A), ADC activation map (B), and BOLD activation maps with the same Z-score threshold (C) or the same number of activated voxels (D) as the ADC activation map, with overlaid WM/GM boundaries (in green) and GM/CSF boundaries (in blue) derived from the anatomical image.

Fig. 2. Maps of the baseline ADC (A), functional ADC change (B), and BOLD signal change (C) in the vicinity of the calcarine fissure, with overlaid WM/GM boundaries (in green) and GM/CSF boundaries (in blue) derived from the anatomical image. The slice is the same as in Figure 1.

Fig. 3. Cortical depth dependence of the baseline ADC (A,D), functional ADC change (B,E), and BOLD signal change (C,F) for a single subject (top) and averaged across three studies (bottom). The error bars represent the standard error of the mean.