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Decreases in ADC observed in tissue areas during activation in cat visual cortex at 9.4 T using high diffusion sensitization

Essa Yacoub¹, Kâmil Uludağ², Kâmil Uğurbil^{1,2}, and Noam Harel¹

¹Center for Magnetic Resonance Research, Department of Radiology, University of Minnesota Medical School, 2021 6th Street S.E., Minneapolis, MN 55455.

²Max-Planck Institute for Biological Cybernetics, Hochfeld Magnetresonanz Zentrum Spemannstr. 41, Tübingen 72076, Germany

Summary

Recent studies in the human visual cortex using diffusion weighted fMRI have suggested that the *apparent* water diffusion coefficient (ADC) *decreases* in contrast to earlier studies which consistently reported ADC *increases* during neuronal activation. The changes, in either case, are hypothesized to provide the ability to improve the spatial specificity of fMRI over conventional BOLD methods. Most recently, the ADC decreases have been suggested as originating from transient cell swelling caused by either shrinkage of the extracellular space or by some intracellular neuronal process which precedes the hemodynamic response. All of these studies have been conducted in humans and at lower magnetic fields, which can be limited by signal to noise ratios (SNR). The low SNR can lead to significant partial volume effects because of the lower spatial resolutions required to attain sufficient SNR in diffusion weighted images. Human studies also have the potential confound of motion. At high magnetic fields, and in animal model studies, these limitations are alleviated. At high fields, SNR increases, tissue signals are enhanced, and signal changes inside the blood are significantly reduced compared to lower fields. In this work, we were able to measure a small but significant ADC *decrease* in tissue areas in conjunction with brain activation in the cat visual cortex at 9.4 T when using highly diffusion weighted images ($b > 1200 \text{ s/mm}^2$) where the intravascular effects are minimal. When using low b-values, delayed *increases* in the tissue ADC during activation were observed. No significant changes in ADC were observed in surface areas containing mostly large vessels for any diffusion weighting. We did not observe any temporal differences in the highly diffusion weighted data compared to BOLD, however, in any case, although the changes may likely be vascular in nature, they are highly localized to the tissue areas.

Keywords

BOLD; Diffusion; fMRI; functional mapping; ADC

Address for Correspondence: Essa Yacoub, Ph.D., Center for Magnetic Resonance Research, 2021 6th Street S.E., Minneapolis, MN 55455, phone: (612) 626-2001; fax: (612)-626-2004, Email: yacoub@cmrr.umn.edu.

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Introduction

Functional MRI BOLD (Blood oxygenation level dependent) signal monitors changes in neural activity in the brain via alterations in cerebral blood flow (CBF), blood volume (CBV) and oxygen consumption (CMRO₂)^{1–5}. This is made possible because the BOLD contrast is sensitive to changes in the tissue concentration and the amount of paramagnetic deoxyhemoglobin which, in turn, reflects perturbations in CBF, CBV and/or CMRO₂. It is well known that in most fMRI studies (especially at field's ≤ 3 T), the source of these signal changes are dominated by large draining veins, which can be up to several millimeters away from the actual site of neural activity^{6–12}. Recently, much attention has been given to diffusion weighted fMRI because of its potential to better localize with neuronal activity and to reflect changes of a non-vascular origin.

The apparent diffusion coefficient (ADC)¹³ has been proposed to monitor water molecular motion using varying magnetic field gradients. Diffusion or intravoxel incoherent motion (IVIM) weighting¹⁴ in MR images imposed by the application of bipolar gradients¹⁵ can selectively attenuate signals from different flowing blood pools, as well as from extra- and intracellular tissue water undergoing diffusion. After changing the amount of diffusion weighting (b-value), the resulting MR signals can be fit to an exponential or multiple exponential functions resulting in an estimation of the ADC. Early studies^{16,17} using diffusion weighted fMRI have suggested that ADC changes during neuronal activation. Furthermore, these changes were predicted to be more spatially correlated with neuronal activation due to the earlier onset of these changes relative to the vascular based BOLD signal¹⁸ as well as distinctive spatial characteristics¹⁹ relative to BOLD. These studies demonstrated repeatedly that the ADC increases during neuronal activation^{18–24}, and were ascribed mainly to the IVIM effect associated with the higher blood flow attained during the activated state. However, more recent studies, conducted in a much higher diffusion weighting regime, reported decreases^{25,26} in apparent ADC with neuronal stimulation. Le Bihan and colleagues have attributed ADC changes observed with high diffusion sensitization to transient cell swelling caused by either a shrinking of the extracellular space or by some intracellular neuronal process which precedes the hemodynamic response²⁶. More recently, this result has been investigated by others^{27,28}, resulting in different findings and subsequent interpretations. Thus, the mechanism of diffusion changes and its polarity (increase or decrease) during activation, as observed via fMRI, is not well understood. The previous human studies, unlike animal studies, can be limited by: signal to noise ratios (SNR) (especially at lower magnetic fields), motion, and significant partial volume effects because of the lower spatial resolutions required for sufficient SNR in diffusion weighted images. Furthermore, signal changes in and around large veins are known to dominate the BOLD signal changes at lower fields. Because of these reasons, the replication, reliability, and general utility of these findings in human studies at lower fields (≤ 3 T) are limited. At high magnetic fields, signal to noise ratios increase, tissue signals are enhanced, and signal changes inside the blood are significantly reduced compared to lower fields. Higher magnetic fields and/or animal models can allow for more reliable and spatially accurate investigations of the fundamental physiological mechanisms associated with neuronal activation. Signal changes in the diffusion weighted BOLD fMRI time course can result from either non-specific intravascular signals (i.e. when the blood T₂ reduces in the presence of deoxyhemoglobin or because of a phase change between tissue and blood when blood occupies a large fraction of a voxel) or more localized extravascular effects, which at high fields using spin echoes reflects dynamic averaging of spins near “tissue” (i.e. small and capillary size vessels (see 29)), as a result of oxygenation, flow, or possibly diffusion related effects. In this work, we attempt to shed light on the apparent discrepancies in the literature on the origin of signal changes in the diffusion weighted fMRI time course using a high resolution cat model at 9.4 T.

Methods

Animal setup

Cats ($n=3$) were prepared as described previously^{30,31}. Briefly, the animals were initially anesthetized intramuscularly with a ketamine (10–25 mg/kg) and xylazine (2.5 mg/kg) cocktail. The animals were intubated and artificially ventilated with isoflurane anesthesia throughout the experiment (1% in a $N_2O:O_2$ mixture of 70:30). Blood pressure, end-tidal CO_2 and body temperature were maintained at normal conditions. The animal's eyes were refracted and focused on the stimulus using corrective contact lenses. The animal was placed in a cradle and restrained in a normal postural position using a custom-designed stereotaxic frame. Visual stimuli consisted of binocular high-contrast square-wave drifting and rotating gratings (0.15 cyc/deg, 2 cyc/s) for 20 s. In order to achieve a maximum amount of activity the drift direction of the gratings was reversed every second and every 2 s the orientation of the gratings was changed in increments of 36° so that a total of 360° of rotation was achieved by the end of a stimulation period.

MRI Methods

All MRI experiments were performed on a 9.4T/31cm (Oxford, UK) magnet equipped with a Varian console (Palo Alto, CA). A 1.4-cm diameter transmit and receive surface coil was used. A coronal slice perpendicular to area 18 (crossing at Horsley-Clark AP2) was used for the functional study (see Fig. 1a). Anatomic images were obtained using a T_1 -weighted 2D TurboFLASH³² sequence. Functional images were obtained using diffusion weighted Hahn-spin-echo (HSE) BOLD sequences. For the HSE BOLD sequence a reduced FOV along the phase encode direction was applied using a selective refocusing pulse^{33–37}. This allowed us to reduce the number of segments needed for the high resolution images. HSE BOLD images were acquired with different amounts of diffusion weighting using Stejskal-Tanner (S-T)¹⁵ gradients applied either simultaneously or separately along the 3 axes. Functional scans were acquired using b-values of: 1, 600, 1200, and 2400 s/mm^2 . Image parameters were: Data matrix = 32×128 , single shot EPI, field of view (FOV) = 0.8×3.20 cm². Slice thickness = 2 mm and TE/TR = 44ms / 2 s. The in plane resolution was 250×250 μm^2 .

Data Analysis

The MRI data were Fourier transformed and analyzed using the Stimulate software³⁸ and Matlab codes (The MathWorks, Inc.). A low pass (temporal) filter was used to remove high frequency noise; no spatial smoothing was applied. Functional time courses were generated by selecting *all pixels* (i.e. pixels were *not* excluded based on statistical thresholds of activation) within regions of interest (ROI) in the tissue areas as well as in the surface vessel areas in each cat (see Fig. 1). Tissue ROIs were centered over the middle cortical layers and expected to primarily contain small vessel or capillary signals³¹. Surface vessel ROIs were dominated by pial surface vessels. The stimulus evoked signal changes were calculated at each of the different diffusion weightings in each of the cats for the respective tissue and vessel ROIs. The measured changes were evaluated as a function of b-value.

Calculation of ADC

ADC can be determined by altering the amount of diffusion weighting, and thus the amount of signal attenuation in a spin echo image. The resulting signals can then be fit according to a mono- or bi-exponential decay function. Depending on the amount of diffusion weighting (i.e. b-value), the images would be sensitive to different parts of the vascular system. Increasing the b-value will result in a suppression of flowing spins beginning with the fastest moving (arterial & venous side) to, when extremely high b-values are reached, slower moving spins in smaller and smaller vessels possibly even near the tissue^{39,40}. In addition to selectively

suppressing different pools of flowing spins, signals throughout the vascular system are modulated by phase shifts consequential to intravoxel incoherent motion¹⁴. ADC and ADC changes were computed using the intravoxel incoherent motion model. The signal intensity for a given echo time in a diffusion weighted image can be described according to the Steskal-Tanner equation:

$$S = S_0 \times \exp(-\gamma^2 g^2 \delta^2 (\Delta - \delta/3) \times \text{ADC}) \quad [1]$$

Where S is the signal intensity in the spin echo image, S_0 is the intensity with no diffusion weighting, and diffusion weighting is referred to as the b -value described by: $b \text{ (sec/mm}^2\text{)} = \gamma^2 g^2 \delta^2 (\Delta - \delta/3)$. Where γ is the gyromagnetic ratio, g is the pulsed gradient amplitude, δ is the pulsed gradient duration, and Δ is the time between the leading edge of the applied gradients. ADC can be calculated from a minimum of 2 b -values according to equation 2:

$$\text{ADC} = \frac{1}{b_1 - b_2} \times \ln \frac{S(b_2)}{S(b_1)} \quad (b_1 < b_2) \quad [2]$$

Where b_1 and b_2 are different diffusion weightings and $S(b_1)$ or $S(b_2)$ represent the MR signal at the respective b -values. Initially, ADC changes were measured using data acquired from all of the different diffusion weightings; subsequently, ADC changes were calculated using only the high b -values (1200 & 2400 s/mm²) or the low b -values (1 & 600 s/mm²) in order to assess differences in observed ADC changes as they might depend on diffusion weighting.

Results

Statistically significant signal changes ($\Delta S/S$) were observed in all 3 cats for spin echo BOLD weighted images at each of the different diffusion weightings as well as with minimal diffusion weighting ($b=1 \text{ s/mm}^2$). A representative series of activation maps from a single cat at the respective diffusion weightings is shown in figure 2. The corresponding background image is the actual diffusion weighted spin echo EPI image from each of the different diffusion weightings. The spatial nature of the functional maps changes as a function of b -value due to both the progressive loss in signal from increased diffusion weightings as well as the different sensitivities of the vascular tree to the applied diffusion gradients. At extremely high b -values the signals tend to be more localized to the gray matter. Note that fMRI activation detection or contrast to noise is reliable even at extremely high b -values of 2400 s/mm².

As mentioned, the analysis of the temporal features of the activation was done using anatomically defined ROIs (see Figure 1) without any spatial biasing. Spatial biasing is routinely done by assuming hemodynamic models and imposing statistical thresholds to selectively choose voxels which behave according to this expected hemodynamic model. This is typically done to reduce the noise in the fMRI time series which is to be analyzed. We found in each cat, using an ROI analysis, a monotonic ($\sim 20\%$) decrease in stimulus evoked BOLD percent changes ($\Delta S/S$) in the *tissue* areas when increasing the b -value from 1 to 1200 s/mm² (see Fig.3b). This decrease is in good agreement with previous work^{36,41}. However, at the highest b -value (2400 s/mm²) an increase in the BOLD tissue signal changes ($\Delta S/S$) is observed in all of the cats, to levels approaching that of the $b=1 \text{ s/mm}^2$ data. This finding is in agreement with the data reported previously by Le Bihan et al²⁶. In contrast, signal changes in the surface vessel ROI did not show any significant dependence on b -value; however, the mean signal change did tend to *decrease* ($p < 0.2$) monotonically with increasing b -values (see Fig 3c). It should be noted that the data from the surface vessel ROI were noisy due to the intrinsically low signal (due to the short T_2 ^{36, 41–43}) inside the blood at this high magnetic field.

Functional ADC ($\Delta\text{ADC}/\text{ADC}$) time courses in the tissue and vessel areas were calculated based on the signal levels (using equations 1–2) and changes from the different diffusion weighted images (Fig. 2) from each of the cats. In the tissue areas, when using a low b-value pair (1 & 600 s/mm²), small and *delayed* increases in the average ADC time course of all cats were observed (~0.30 %) (see Fig 4 - red). When using the high b-value pair (1200 & 2400 s/mm²) to calculate ADC, *earlier* and significant *decreases* (~-0.45 %) were observed following activation in the average ADC time courses (see Fig 4- blue). If all b-values are used, very small, insignificant changes in ADC were observed during activation (Fig. 4b). In the surface vessel ROIs, while noisy, the ADC changes (with either low or high b-values) were small, but did tend to increase during activation ($p < 0.2$). We did not observe any significant differences in the onset time between any of the diffusion weighted data compared to the BOLD ($b=1$ s/mm²) data. Note, however, that subtle differences in the onset time may be difficult to detect with limited temporal resolution (2 s) as used in this study. However, on average, there was a shift in the time to peak between the low b-value (peaking later) and the highest b-value (peaking earlier), resulting in differences in the total duration of the response.

Discussion

In this work, we investigated the dependence of stimulus evoked signal changes under the presence of low and high Stejskal-Tanner gradients (i.e., diffusion weighting) in a HSE BOLD sequence in the cat visual cortex during visual stimulation at 9.4 T. The study was conducted with high spatial resolution and allowed for a clear distinction between tissue regions, areas containing mainly capillary size vessels, versus the cortical surface, areas containing mainly large pial vessels. We found that the polarity of the observed ADC changes depend on the amount of diffusion weighting that is used. In the tissue ROI, during activation, an *increase* in ADC is observed if relatively low b-values (1– 600 s/mm²) are used to calculate ADC. In contrast, if higher (1200 – 2400 s/mm²) b-values are used to calculate ADC, the exact same pixels exhibit a *decrease* in ADC during activation.

In the literature, there are conflicting observations regarding changes associated with diffusion weighted fMRI following neuronal stimulation, which are accompanied by conflicting explanations regarding the physiological origin of these changes. We attempted to address some of these discrepancies in this study.

ADC changes during activation: Vascular or Neuronal

It has been reported that ADC changes exist and can be observed following sensory stimulation using diffusion weighted fMRI techniques^{16,17}. Initial reports^{18–23} demonstrated *increases* (as high as 15%) in the ADC that correlated with an increase in brain activity. These studies were done using relatively low diffusion weightings ($b < 500$ s/mm²) at magnetic fields ≤ 4 T. The significance and utility of the observed ADC changes were investigated extensively and it was found that the spatial and temporal nature of the ADC changes during activation were significantly distinct from conventional BOLD signals^{18–20}. The ADC changes would consistently precede BOLD changes on the order of about 1 second. This is important because the BOLD signal at these field strengths originates mostly from the venous side and thus ADC changes, similar to arterial spin labeling methods (ASL), might increase the relative contribution of signals originating from arteries (or even capillaries), and ultimately provide an fMRI contrast mechanism with improved spatial localization of brain activation over conventional BOLD signals. More recently^{25, 26}, operating in a *different*, much higher, diffusion regime (maximum b-value of 2400 s/mm² applied simultaneously in x, y, z directions), small *decreases* (~ 1%) in the ADC during brain activation were observed. At these high b-values it would be expected that signal changes originate *solely* from the extravascular space as the intravascular signal would be eliminated. The ADC decreases, similar to the

increases, were found to precede the BOLD signals on the order of seconds. Le Bihan and colleagues attributed this finding to being cellular in nature. The changes were hypothesized to reflect neuronal activation induced cell swelling or membrane expansion. More specifically, it was recently suggested²⁶ that there was a small dilation of the slow water diffusing pool and with that a decrease in volume of the fast water diffusion pool.

In our data, more pronounced when averaging across cats (See Fig.3a), a difference in the time to peak of the response, *not* the onset time as demonstrated in the human studies, and a subsequent difference in the duration (width) of the response was observed (with the high b-value data being narrower and peaking sooner). Also, in contrast to the mentioned human studies, Miller et al²⁷ (a human study) did not observe any *temporal* differences between the diffusion weighted functional signal changes and the BOLD signal changes. In human studies (i.e.25,26), the signal to noise of the fMRI data is limited especially when employing high b-values and the observed changes/temporal differences are generally only visible when averaging across subjects and not in single studies as demonstrated here, making it difficult to quantify such subtle effects. Furthermore, statistical biasing/isolation of voxels is normally used in the analysis to identify pixels behaving similar to a predicted hemodynamic response model, which reduces the noise in the fMRI time series but also likely introduces spatial biases. In this work, no statistical thresholding was imposed, but rather ROI-based analyses were done which did not show any significant differences in onset times. An absence of temporal differences is important because it would suggest that 1) the observed signal changes might be vascular (not cellular) in nature and 2) if the temporal dynamics are similar to BOLD, the potentially vascular changes would originate from the capillaries or small vessels. However, whether the high diffusion response is regulated by neuronal or vascular processes, the change in diffusion weighting inherently changes the vascular contributions to the signal^{14,15} (i.e. selectively reducing the signals from flowing blood) and likely introduces differences in the temporal features. In our data, when averaging across cats (See Fig.3a), a difference in the time to peak of the response, *not* the onset time, and therefore a subsequent difference in the duration (width) of the response was observed (with the high b-value data being narrower and peaking sooner). We do not believe that this finding is evidence for a neuronal nature to the response, as we do not see any significant differences in the onset time, while the differences in the evolution of the response could be explained by the different vascular sources present at the respective diffusion weightings and the subsequent temporal evolution of the different vascular components.

A more straightforward way to test the vascular nature of the signal changes, while avoiding the constraining SNR limited task of detecting significant subtle differences in timing, was done by Miller et al²⁷ using a hypercapnia model. Hypercapnia disentangles the vascular response from neuronal activity: it induces increases in blood flow while having a minor effect on neuronal activity. For that reason, if the observed diffusion weighted functional changes were neuronal in nature (i.e. not vascular), one would not expect to see ADC changes under hypercapnia conditions. Miller et al. found that the increases in signal changes with increasing diffusion weighting persisted under hypercapnia conditions, implicating a significant vascular nature to the response. However, the nature of a potential vascular response is unclear, considering there is likely to be a minimal amount of blood signal remaining at these extremely high b values. Finally, another study using the cat model at 9.4 T²⁸, did not observe *any* tissue related ADC changes during activation. The discrepancy could be easily explained by the parameters used; the maximum b-value used in that study was 800 s/mm²; here, the observed decreases in ADC in tissue areas were significant *only* when the high b-value pair (1200 & 2400 s/mm²) was used to calculate ADC. Also, a consistent finding between our study and²⁸ was that when the relative intravascular component increased either with shorter echo times²⁸ or lower b-values (this study) the subsequent observed ADC changes were *increases*, not decreases.

ADC increases versus decreases

Several studies^{25–27}, in addition to this work (see Fig 3b), have reported similar findings of an increasing BOLD ($\Delta S/S$) signal when monitoring functional signal changes under the presence of high diffusion weightings. The finding of a possible phase change in the diffusion weighted signal changes (i.e. ADC increase versus no change or decrease) appears to have some consistency between all the studies mentioned above as well as with this work. That is, at lower fields, shorter echo times¹ or smaller diffusion weightings, where the *intravascular* component or the blood signal is expected to be larger, the observed changes in ADC, which correlate with brain activation, are *increases*; however, when the relative contribution of the intravascular signal is decreased either by going to higher fields and/or longer echo times or increasing the b-value, these ADC signal changes become *minimal* and subsequently exhibit (*earlier*) *decreases* during brain activation (see cartoon in Fig 5).

This relative *difference* in the intravascular contributions to the data may explain why some studies reported increases, while yet others reported decreases, and why even some report no changes in the ADC during activation.

Our data indicate that the origin of this relative signal change increase at the high b-value and subsequent decrease in ADC during activation must be extravascular in nature as the intravascular component of the BOLD signal at 9.4 T coupled with such strong diffusion gradients, is negligible, while the increases at lower b-values may be the result of residual intravascular or blood related effects. Furthermore, to account for non-optimal directions of blood flow, where application of simultaneous S-T gradients may not suffice, we in addition ran experiments with S-T gradients applied non-simultaneously on each axis. These experiments resulted in similar findings. The finding at high b-values was observed in the tissue areas and absent in the vessel areas. Although originating in the tissue, we believe that a strong vascular contribution to this effect is more likely based on the lack of significant temporal differences coupled with the recent hypercapnia data²⁷. Such a mechanism, however, for example a CBV increase which might constrain tissue ADC, changing the extravascular BOLD signal, is not obvious and would be difficult to investigate. Irrespective of the physiological mechanism associated with the ADC decreases linked to brain activation, we observed this response only in the tissue areas, using a high field, high resolution cat model coupled with extremely high b-values - implicating a highly spatially specific nature to the signal changes. This *high* specificity, however, may not necessarily remain when relatively larger intravascular signal persist and ADC increases are observed.

Our study was not well suited to address small (< 2s) temporal differences between diffusion weighted fMRI and BOLD time courses. This would require higher temporal resolution while maintaining a sufficient amount of sensitivity to detect such subtle changes in timing without imposing statistical biases. Therefore, we cannot say per se whether the origin of the ADC *decreases* are vascular or neuronal in nature, only that if they are vascular they are extravascular and they are likely localized to the tissue areas while the observed ADC *increases* appear to be correlated with the relative amount of intravascular signal present and likely less localized to the tissue areas.

Finally, one other scenario, which we have not discussed as a possible explanation for the observed ADC changes, is the interaction of 2 gradient fields. That is, the susceptibility induced gradient field during activation interacting with the diffusion gradients. This effect may be significant only when the diffusion gradients are extremely large as they are at high b-values.

¹Shorter echo times will in general reduce the relative amount of intravascular signal (see simulation in Duong et al., 2003) except at 1.5 T where the tissue T₂ is shorter than the blood T₂

Further studies are needed to investigate whether these effects are responsible for the observed ADC changes at high b-values.

Conclusions

In this work, we were able to measure a small but significant ADC decrease in tissue areas in conjunction with brain activation in the cat visual cortex at 9.4 T when using highly diffusion weighted images ($b > 1200 \text{ s/mm}^2$) where the intravascular effects are minimal. When using low b-values, where the intravascular effects are small (10 – 20 %) but persist, small delayed increases in the ADC during activation were observed.

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Figure 1. Regions of interest (ROIs) were selected in the tissue and surface vessel areas (green-vessel ROI, red-tissue ROI) in a representative cat. Data analyses were done using all voxels included in these ROIs (scale bar = 1 mm).

Figure 2.
BOLD activation maps from different b-values overlaid on diffusion weighted spin echo EPI images from a representative cat.

Figure 3.

Averaged BOLD time courses in the tissue at each of the b-values (a), and plotted on the right (b–c) are the normalized BOLD changes at each of the b-values for each of the cats (cat 1 (red), cat 2 (blue), and cat 3 (green); average (dashed)) for the tissue and surface vessel ROIs, respectively. In all 3 cats the BOLD signal in the tissue decreases as a function of b-value until the highest b-value is reached (2400 s/mm²) where the BOLD signal change recovers to a value similar to the lowest b-value (1 s/mm²). No significant changes as a function of b-value were observed in the vessel ROIs. (Green box in (a) indicates the stimulus)

Figure 4.

a) ADC calculated changes for low (1–600 s/mm²) and high (1200–2400 s/mm²) b-values, in the parenchyma, averaged over 3 cats, according to equations 1&2. b) ADC changes calculated for all b-values (1– 2400 s/mm²) (Green box indicates the stimulus).

Figure 5.

Graphical illustration of the observed ADC changes consistent with our findings as well as previous reports on ADC changes during activation. Green represents the relative amount of extravascular compared to intravascular (red) signal contributing to the BOLD signal for a given TE, b-value and magnetic field. If the TE, diffusion weighting (b), or magnetic field (B_0) (because of blood T_2 changes) are changed, the relative amount of intravascular versus extravascular signal also changes and with that the observed ADC changes and the sign of those changes (vertical axis) also change.