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The Effect of Microcirculatory Flow on Oscillating Gradient Diffusion MRI and Diffusion Encoding with Dual-Frequency Orthogonal Gradients (DEFOG)

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Abstract

Purpose—We investigated the effect of microcirculatory flow on oscillating gradient spin echo (OGSE) diffusion MRI at low b-values and developed a diffusion preparation method called diffusion encoding with dual-frequency orthogonal gradients (DEFOG) to suppress the effect.

Methods—Compared to conventional OGSE sequences, DEFOG adds a pulsed gradient that is orthogonal to the oscillating gradient and has a moderate diffusion weighting (e.g., 300 s/mm²). In vivo MRI data were acquired from adult mouse brains ($n = 5$) on an 11.7 Tesla scanner, with diffusion times from 23.2 to 0.83 ms and b-values from 50 to 700 s/mm².

Results—Apparent diffusion coefficients (ADCs) measured using a conventional OGSE sequence at low b-values ($< 200 \text{ mm}^2/\text{s}$) were significantly higher than those measured at moderate b-values ($> 300 \text{ mm}^2/\text{s}$), potentially due to contributions from microcirculatory flow. In comparison, OGSE ADCs measured using the DEFOG method at low b-values were comparable to those measured at moderate b-values. The effect of microcirculatory flow on diffusion signals was diffusion time-dependent, and this dependency may reflect the capillary geometry and blood flow velocity in the mouse cortex.

Conclusion—Microcirculatory flow affects OGSE diffusion MRI measurements at low b-values, and this effect can be suppressed using the DEFOG method.

Keywords

diffusion MRI; oscillating gradient; dual-frequency orthogonal gradients; pseudo-diffusion; microcirculatory flow; mouse brain

INTRODUCTION

Oscillating gradient spin echo (OGSE) diffusion MRI has recently drawn attention due to its potential to distinguish tissue microstructures at varying length scales (1,2). The technique

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uses oscillating gradients of different frequencies to sample the temporal diffusion spectrum $D(\omega)$, which is the Fourier transform of the water-molecule velocity autocorrelation function (3). The shape of $D(\omega)$ has been linked to several microstructural properties of tissue, such as cell size, membrane permeability, and surface-to-volume ratio (4–6). Phantom and animal studies have demonstrated the unique ability of OGSE diffusion MRI to characterize tissue microstructures under normal (7–14) and pathological conditions, such as in brain tumor (15,16) and stroke (1,9,17). Applications of this technique in human studies, however, are often hindered by the limited gradient strength on current clinical MR systems (< 100 mT/m). Even at relatively low-oscillating frequencies of 50 to 60 Hz, the effective b-value, or diffusion weighting, in previous human studies reached only 200 to 300 s/mm² (9,18) because the b-value decreases rapidly with increasing oscillating frequency ($b \propto 1/f^3$) if the gradient magnitude and timing remain constant (2).

With the limitation on b-value, both the sensitivity of OGSE diffusion MRI to tissue microstructures and the range of available oscillating frequencies are reduced. More importantly, signal attenuation due to microcirculatory flow, which mimics diffusion and reflects blood flow in the capillaries and some other small vessels (20,21), must be considered at low b-values to avoid biases in the estimated apparent diffusion coefficient (ADC). This microcirculatory flow effect had been shown in diffusion signals, measured using conventional pulsed gradient spin echo (PGSE) sequences at low b-values (< 200 – 300 s/mm²), and can be separated out by fitting appropriate models to ADC measurements acquired at multiple b-values (21–25). Questions about a similar effect on OGSE diffusion MRI have been recently raised (19) but have not yet been examined. In this study, we investigated how microcirculatory flow affects OGSE measurements at low b-values ($b < 300$ s/mm²) and propose a new diffusion preparation method that combines orthogonal pulsed and oscillating gradient waveforms to suppress this effect. We named the method diffusion encoding with dual-frequency orthogonal gradients (DEFOG).

THEORY

Diffusion Encoding with Dual-Frequency Gradient Waveforms

The signal attenuation measured by OGSE diffusion MRI can be described as

$$\ln \left(\frac{S}{S_0} \right) = - \frac{1}{\pi} \int_0^\infty F(\omega) D(\omega) F(-\omega) d\omega, \quad [1]$$

where S and S_0 are diffusion-weighted and nondiffusion-weighted signals, respectively, and ω is the oscillating frequency (2). $F(\omega)$ is the gradient modulation spectrum (1), defined as

$F(\omega) = \frac{\gamma}{2\pi} \int_{-\infty}^\infty \left(\int_0^t G(\tau) d\tau \right) e^{-i\omega t} dt$ (3), where γ is the gyromagnetic ratio and $G(t)$ is the diffusion gradient waveform. Because $G(t)$ is a real-value function here, we have $F^\dagger(\omega) = F(-\omega)$ and $|F(\omega)|^2 = |F(-\omega)|^2$, where $\dagger$ denotes a complex conjugate. $|F(\omega)|^2$ is the gradient

modulation power spectrum (19) and $b = \frac{1}{\pi} \int_0^\infty |F(\omega)|^2 d\omega$.

When two or more finite gradient waveforms are simultaneously applied, the $F(\omega)$ of the combined gradient waveform is simply the sum of the individual gradient modulation spectra because both the Fourier transform and the time integral are linear operations. If the gradient modulation power spectra of the simultaneously applied gradient waveforms all have limited bandwidths, with no overlap among them in the frequency domain, the signal attenuation due to the combined diffusion gradient waveforms becomes

$$\ln \left(\frac{S}{S_0} \right) = - \frac{1}{\pi} \sum_{i=1}^M \int_0^{\infty} F_i(\omega) D_i(\omega) F_i(-\omega) d\omega, \quad [2]$$

where M is the number of simultaneously applied gradient waveforms; $F_i(\omega)$ is the gradient modulation spectrum of the gradient waveform $G_i(t)$; and $D_i(\omega)$ is the temporal diffusion spectrum along the direction of $G_i(t)$, for $i = 1, \dots, M$. For the conventional pulsed gradient waveforms and commonly used modified cosine oscillating gradient waveforms (1), Eq. [2] can be expressed in a simplified form as

$$\ln \left(\frac{S}{S_0} \right) = - \sum_{i=1}^M b_i \cdot D_i(\omega_i), \quad [3]$$

where b_i is the b-value of $G_i(t)$ and ω_i is the peak frequency of $|F_i(\omega)|^2$.

When two gradient waveforms, $G_1(t)$ and $G_2(t)$ with b-values of b_1 and b_2 and an angle θ between their directions, are applied simultaneously, the b-value of the combined gradient waveform can be calculated using the following equation,

$$b_{combined} = b_1 + b_2 + 2\gamma^2 \int_0^{TE} \left[\int_0^t G_1(\tau) d\tau \cdot \int_0^t G_2(\tau) d\tau \cdot \cos(\theta) \right] dt, \quad [4]$$

where TE is the echo time. Because the last term in Eq. [4] is not necessarily zero, in general the b-value of the combined gradient waveform does not equal $b_1 + b_2$, and similarly the $|F(\omega)|^2$ of the combined gradient waveform does not equal the sum of the modulation power spectra of the two gradient waveforms, $|F_1(\omega)|^2$ and $|F_2(\omega)|^2$ (Figs. 1a and 1b). One exception is when $G_1(t)$ and $G_2(t)$ are orthogonal to each other; that is, $\cos(\theta) = 0$, the b-value of the combined gradient waveform equals $b_1 + b_2$, and $|F(\omega)|^2$ of the combined gradient waveform equals $|F_1(\omega)|^2 + |F_2(\omega)|^2$ (Figs. 1c and 1d).

Signal Attenuation Under the DEFOG Scheme

The signal attenuation due to microcirculatory flow can be incorporated into the diffusion signal equation as

$$\frac{S}{S_0} = f \cdot H(b, \Delta, l, v, D_{blood}) + (1 - f) e^{-b \cdot D}, \quad [5]$$

where $f \in [0,1]$ is the fraction of signal attenuation associated with microcirculatory flow, and D is the apparent diffusion coefficient of tissue water molecules. Note that the signal attenuation due to microcirculatory flow is represented by a general function, $H(b, \Delta, l, v, D_{blood})$, where l and v are the average length of capillary segments and the average velocity of blood flow in the capillary bed, respectively; Δ is the diffusion time; and D_{blood} is the apparent diffusion coefficient of water molecules in the blood. When Δ is long enough for the microcirculatory flow to pass through multiple vascular segments (model 1 as described in (21)), we have $H_1(b, \Delta, l, v, D_{blood}) = e^{-b \cdot D^*}$ for both pulsed and oscillating gradient

waveforms, where D^* is the so-called pseudo-diffusion coefficient and $D^* = \frac{l \cdot v}{6} + D_{blood}$ (21). When Δ is short enough that most microcirculatory flow remains in one vascular segment during Δ (model 2, as described in (21)), we have

$H_2(b, \Delta, l, v, D_{blood}) = |\sin(c \cdot v) / (c \cdot v)| \cdot e^{-b \cdot D_{blood}}$, where $c = \gamma \int_0^{\Delta} \tau \cdot G(\tau) d\tau$. For flow-compensated pulsed gradient waveforms, as described in (26) and the cosine-trapezoid oscillating gradient waveforms (19) used in this study, their first-order moment is zero; that is, $c = 0$, and we have $H_2(b, \Delta, l, v, D_{blood}) = e^{-b \cdot D_{blood}}$ for microcirculatory flow that fits model 2. For general cases, we assume that a fraction (f_1) of the signal comes from microcirculatory flow that fits model 1; another fraction (f_2) comes from microcirculatory flow that fits model 2; and the rest ($1 - f_1 - f_2$) comes from tissue water. Eq. [5] becomes

$$\frac{S}{S_0} = f_1 \cdot H_1(b, \Delta, l, v, D_{blood}) + f_2 \cdot H_2(b, \Delta, l, v, D_{blood}) + (1 - f_1 - f_2) e^{-b \cdot D}. \quad [6]$$

For a cosine-trapezoid oscillating gradient waveform, $G_1(t)$, with an oscillating frequency of ω_1 and a b-value of b_1 , we have the following signal attenuation equation,

$$\frac{S_{G_1}}{S_0} = f_1 \cdot e^{-b_1 \cdot D^*(\omega_1)} + f_2 \cdot e^{-b_1 \cdot D_{blood}(\omega_1)} + (1 - f_1 - f_2) \cdot e^{-b_1 \cdot D_1(\omega_1)}, \quad [7]$$

where $D^*(\omega_1)$ and $D_1(\omega_1)$ are the pseudo-diffusion coefficient and ADC of tissue water molecules measured with $G_1(t)$, respectively. We use $D_{blood}(\omega_1)$ in Eq. [7] because D_{blood} is also dependent on diffusion time, or in this case, oscillating frequency (27).

For a conventional pulse gradient waveform, $G_2(t)$, with a b-value of b_2 , we have the following signal attenuation equation,

$$\frac{S_{G_2}}{S_0} = f_1 \cdot e^{-b_2 \cdot D_{PGSE}^*} + f_2 \cdot \left| \frac{\sin(c_2 v)}{c_2 v} \right| \cdot e^{-b_2 \cdot D_{blood, PGSE}} + (1 - f_1 - f_2) \cdot e^{-b_2 \cdot D_{PGSE}}, \quad [8]$$

where D_{PGSE}^* , $D_{blood, PGSE}$, and D_{PGSE} are the pseudo-diffusion coefficient and ADC of blood and tissue water measured with $G_2(t)$, respectively. In Eq. [8], c_2 is the first-order moment of $G_2(t)$.

By comparing diffusion signals acquired with an OGSE gradient waveform, $G_1(t)$, and an orthogonal PGSE gradient waveform, $G_2(t)$ —as in the DEFOG scheme—to signals acquired with $G_2(t)$ only, we have from Eqs. [3], [7], and [8]

$$\frac{S_{G_1+G_2}}{S_{G_2}} = \frac{f_1 \cdot e^{-b_1 \cdot D^*(\omega_1)} \cdot e^{-b_2 \cdot D_{PGSE}^*} + f_2 \cdot e^{-b_1 \cdot D_{blood}(\omega_1)} \cdot \left| \frac{\sin(c_2 v)}{c_2 v} \right| \cdot e^{-b_2 \cdot D_{blood, PGSE}} + (1 - f_1 - f_2) \cdot e^{-b_1 \cdot D_1(\omega_1)} \cdot e^{-b_2 \cdot D_{PGSE}}}{f_1 \cdot e^{-b_2 \cdot D_{PGSE}^*} + f_2 \cdot \left| \frac{\sin(c_2 v)}{c_2 v} \right| \cdot e^{-b_2 \cdot D_{blood, PGSE}} + (1 - f_1 - f_2) \cdot e^{-b_2 \cdot D_{PGSE}}}. \quad [9]$$

In Eq. [9], we assume that the D^* is isotropic. This assumption is supported by the random orientations of the capillary vessels in the mouse cortex (28). As b_2 increases, the first two terms in the numerator and denominator in Eq. [9], which are related to microcirculatory flow, approach zero at a faster rate than the last term, which reflects tissue water, because 1)

$D_{PGSE}^* \gg D_{PGSE}$; 2) $\left| \frac{\sin(c_2 v)}{c_2 v} \right| \rightarrow 0$ for large b_2 (Table 1); and 3) f_1 and f_2 are usually less than 10% in the brain (21). When the b_2 is large enough, we can divide both numerator and denominator in Eq. [9] by $(1 - f_1 - f_2) \cdot e^{-b_2 \cdot D_{PGSE}}$ and have

$$\frac{S_{G_1+G_2}}{S_{G_2}} \approx e^{-b_1 \cdot D_1(\omega_1)}. \quad [10]$$

From Eq. [10], $D_1(\omega_1)$ can be estimated using the following equation:

$$D_1(\omega_1) = -\frac{1}{b_1} \ln \left(\frac{S_{G_1+G_2}}{S_{G_2}} \right). \quad [11]$$

The condition that b_2 is large enough can be easily achieved because $G_2(t)$ is a pulsed gradient waveform. This means that by combining a pulsed gradient waveform with a moderate-to-high diffusion weighting, for example, $b = 300 \text{ s/mm}^2$, and an oscillating gradient waveform with a relatively weak diffusion-weighting that is orthogonal to the pulsed gradient waveform, the apparent diffusion coefficient along the direction of the oscillating gradient can be estimated using Eq. [11] with the contribution from microcirculatory flow suppressed.

METHODS

Image Acquisition

In vivo MRI experiments were performed on healthy C57BL/6J adult mice (male, 3 months old, number of animal (n) = 5) using an 11.7 Tesla (T) horizontal NMR spectrometer

(Bruker Biospin, Billerica, MA; maximum gradient strength = 760 mT/m) with a 72-mm diameter quadrature volume transmitter coil and a 10-mm diameter receive-only planar surface coil. T₂-weighted images were acquired using the rapid acquisition with relaxation enhancement sequence with the following parameters: effective TE/repetition time (TR)=50/2,000 ms; echo train length = 8; field of view (FOV) = 16 mm × 16 mm; in-plane resolution = 0.08 mm × 0.08 mm; and five slices with a slice thickness = 1 mm (the second slice cut through the anterior commissure). All experimental procedures were approved by the Animal Care and Use Committee at the Johns Hopkins University School of Medicine, Baltimore, Maryland.

The DEFOG diffusion MRI sequence used in this study consisted of the cosine-trapezoid oscillating gradients (19) along six directions, as listed in gradient table B1 below (the three numbers in each row are the x, y, and z components), and orthogonally orientated pulsed gradients along the directions, as listed in gradient table B2. The gradient tables can be expanded to include more directions.

$$B1 = \frac{1}{\sqrt{2}} \begin{bmatrix} 1 & 1 & 0 \\ 0 & 1 & 1 \\ 1 & 0 & 1 \\ 1 & -1 & 0 \\ 0 & 1 & -1 \\ -1 & 0 & 1 \end{bmatrix} \text{ and } B2 = \begin{bmatrix} 0 & 0 & 1 \\ 1 & 0 & 0 \\ 0 & 1 & 0 \\ 0 & 0 & 1 \\ 1 & 0 & 0 \\ 0 & 0 & 1 \end{bmatrix}.$$

Two sets of diffusion experiments were performed:

1. ADC measurements at multiple b-values. All diffusion MRI data were acquired with the following parameters: TE = 32 ms; TR = 2,000 ms; four-segment echo-planar imaging readout; two signal averages; the same FOV and slice arrangement as the T₂-weighted image; and an in-plane resolution = 0.17 mm × 0.17 mm (interpolated to 0.08 mm × 0.08 mm). Conventional PGSE diffusion MRI experiments were performed with $\delta = 10$ ms, $\tau = 13.2$ ms, the diffusion directions listed in gradient table B2, and b-values from 50 to 1,000 s/mm². Conventional OGSE diffusion MRI experiments were performed with two 10-ms trapezoid cosine waveforms before and after the refocusing pulse; with the six diffusion directions, as listed in gradient table B1; and with an oscillating frequency of 100 Hz (number of cycles $N = 1$, equivalent diffusion time = 2.5 ms, and $b = 50\text{--}700$ s/mm²) and 200 Hz ($N = 2$, equivalent diffusion time = 1.25 ms, and $b = 50\text{--}300$ s/mm²). DEFOG experiments were performed with two settings: 1) the 10-ms trapezoid cosine waveforms, as in the conventional OGSE scans ($b = 50\text{--}700$ s/mm² for 100 Hz and $b = 50\text{--}300$ s/mm² for 200 Hz, the diffusion directions in table B1) and an orthogonal pulsed gradient waveform ($\delta = 10$ ms, $\tau = 13.2$ ms, $b = 300$ s/mm², the diffusion directions listed in table B2); and 2) only the pulsed gradient waveform ($\delta = 10$ ms, $\tau = 13.2$ ms, $b = 300$ s/mm², the diffusion directions listed in table B2). To test the sequences, the same experiments were first performed on an agarose gel phantom (4% concentration by weight) using a 17.6 T vertical bore NMR spectrometer (Bruker

Biospin; maximum gradient strength of 1,500 mT/m), which was equipped with a temperature control unit with feedback air heating and cooling systems provided by the manufacturer to maintain the temperature of the phantom at 32 °C. Images were acquired using a 20-mm diameter quadrature volume transmitter-receiver coil and the imaging parameters listed above. Each experiment was repeated three times.

2. ADC measurements at multiple diffusion times (0.83, 1.0, 1.25, 1.67, 2.5, 5.0, and 23.2 ms) and a fixed b-value of 300 s/mm². Data were acquired using a pair of 20-ms diffusion gradient waveforms in order to measure high oscillating frequency at reasonable b-values. The image parameters were: TE = 52 ms, TR = 2,000 ms, four signal averages, and the same imaging resolution as in (A). DEFOG-OGSE data were acquired with oscillating frequencies of 50 Hz, 100 Hz, 150 Hz, 200 Hz, 250 Hz, and 300 Hz (the equivalent diffusion times are 5 ms; 2.5 ms; 1.67 ms; 1.25 ms; 1.0 ms; and 0.83 ms, respectively), the diffusion directions listed in gradient table B1, and an orthogonal pulsed gradient waveform ($\delta = 20$ ms and $\tau = 23.2$ ms) with $b = 300$ s/mm² and the diffusion directions listed in gradient table B2. PGSE data were acquired with $\delta = 20$ ms, $\tau = 23.2$ ms, $b = 300$ s/mm², and the diffusion directions listed in gradient table B2.

Data Analysis

The ADCs measured using the DEFOG sequence were obtained according to Eq. [11] for each set of gradients and then were averaged over the six diffusion directions in gradient table B1. Diffusion tensor data were also acquired using the DEFOG sequence. First, D_j , the diffusivity along the j^{th} diffusion direction, was calculated according to Eq. [11]. Diffusion tensor was then estimated using least squares fitting from $[D_1, D_2, \dots, D_n]$ ($n = 6$) in Matlab (www.mathworks.com; MathWorks, Natick, MA). Images were generated from the diffusion tensor data, fractional anisotropy (FA), and directionally encoded color (DEC) map (29).

Regions of interest (ROIs) were defined for the cortex, and cerebral peduncle on the FA images acquired using the conventional PGSE sequence. We focused on these two structures because the mouse cortex has sufficient cerebral perfusion and relatively homogenous microstructures, whereas the cerebral peduncle is a large white matter tract and has lower capillary density than the cortex (30). The signal-to-noise ratio (SNR) of an image was calculated as the mean signal in the predefined ROI divided by the standard deviation (SD) of the background signals. The SNR of the cortical region was measured in the nondiffusion-weighted image of each subject. From the five mice, the SNR of the cortical region was 103.3 ± 16.3 ($n = 5$) in experiment A and 87.2 ± 17.1 ($n = 5$) in experiment B. The coefficient of variation (CV), which is the ratio between the standard deviation of the ADC values ($n = 5$) and the mean ADC, was measured in the cortical ROI. Because the data were obtained from different subjects, the estimated standard deviation included both intersubject and intrasubject variations; estimated CVs were probably higher than the CVs measured from repeated experiments in the same subject, which was not feasible given the long imaging time required to acquire images at multiple frequencies and b-values.

Data from the ROIs were presented as mean value $\pm$ standard error of the mean (SEM) ($n = 5$). Statistical analysis was performed to evaluate the differences between OGSE and DEFOG measurements and the effects of b-value on these measurements using two-way analysis of variation (ANOVA) with repeated measures in Graph-Pad Prism (www.graphpad.com/scientific-software/prism; GraphPad Software, Inc., La Jolla, CA). To correct for multiple comparisons, we used Bonferroni posttests, which apply a modified significance threshold that was determined by dividing the original significance threshold by the number of comparisons. The linear regression of ADC values against the oscillating frequency was also performed in GraphPad Prism (GraphPad Software, Inc.); and the R^2 , P value, and slopes of the linear regression were obtained. A paired t test was used to compare the slopes of ADCs measured with the OGSE and the DEFOG sequences. $P < 0.001$ was considered highly significant; $P < 0.01$ was considered significant; and $P > 0.05$ was considered nonsignificant.

RESULTS

In phantom experiments, ADCs measured using conventional PGSE, OGSE, and DEFOG OGSE methods did not show a significant difference for b-values that ranged show a significant difference for b-values that ranged from 50 to 700 s/mm² (Table 2) ($P=0.68$ and 0.40 for 100 Hz and 200 Hz, respectively). Figure 2a shows in vivo ADC maps acquired from a representative mouse brain using conventional PGSE, OGSE, and the DEFOG sequences at two b-values. The PGSE and OGSE ADC maps at $b=200$ s/mm² had higher ADC values overall than the DEFOG ADC maps acquired at $b=200$ s/mm², and the difference mostly disappeared when the b-value was raised to 500 s/mm². Figure 2b shows the corresponding T₂-weighted and FA images with the defined ROIs in the cortex and cerebral peduncle overlaid on the FA image. The intensity profile in Figure 3 indicated that the whole-brain ADC value distribution obtained with DEFOG at $b = 200$ s/mm² was similar to that acquired with OGSE at $b = 500$ s/mm². The CV of ADC values measured in the cortical ROIs showed higher variations in the OGSE measurements compared to the DEFOG measurements at low b-values (Table 3).

In the cortex, the PGSE and OGSE ADC values at low b-values (e.g., 50 s/mm²) were significantly higher compared to those at moderate b-values (> 300 s/mm²) (Fig. 4a). In comparison, the DEFOG ADC values (dashed lines in Fig. 4a) showed no significant difference over the range of b-values ($P = 0.1$ and 0.7 for 100 Hz and 200 Hz, respectively; one-way ANOVA). Two-way ANOVA analysis of the OGSE measurements at different b-values showed significant differences between the OGSE and DEFOG measurements ($P < 0.0001$) at both the 100 Hz and the 200 Hz oscillating frequency. Bonferroni posttests showed that the differences between OGSE and DEFOG ADC values were significant at b-values of 50 to 150 s/mm² for the 100 Hz measurements and at b-values of 50 to 100 s/mm² for the 200 Hz measurements. For b-values greater than 200 s/mm², neither the OGSE nor the DEFOG measurements showed a significant change with increasing b-values, and the DEFOG and OGSE ADC curves arrived at similar values. In the cerebral peduncles, ADC values showed no apparent change as the b-value decreased, and no significant difference between OGSE and DEFOG ADC values was detected (Fig. 4b), likely due to the reduced capillary density in white matter tracts.

We then compared mouse cortical ADCs acquired using the conventional PGSE/OGSE and DEFOG sequences at b -values of 100 s/mm^2 and 150 s/mm^2 , respectively (Figs. 5a–b). As expected, the PGSE/OGSE ADC values were significantly higher than the DEFOG ADC values at each b -value, and both OGSE and DEFOG ADC values increased with increasing frequency. Interestingly, the differences between OGSE and DEFOG ADC measurements measured at low b -values gradually decreased as the frequency increased. We found that the DEFOG ADC measurements increased with increasing frequency at a significantly higher rate than the OGSE ADC measurements ($P = 0.02$ for $b = 100 \text{ s/mm}^2$, $P = 0.03$ for $b = 150 \text{ s/mm}^2$; $n = 5$). For example, at a b -value of 100 s/mm^2 , the linear regression between frequency and DEFOG ADCs had a slope of $6.53 \times 10^{-7} \text{ mm}^2$ ($R^2 = 0.64$, $P = 0.0003$) (solid line in Fig. 5a); whereas, for the OGSE/PGSE ADCs, the slope of the linear regression was $2.17 \times 10^{-7} \text{ mm}^2$ ($R^2 = 0.09$, $P = 0.29$) (dashed line in Fig. 5a).

Using the DEFOG sequence, we were able to measure the ADC values of the mouse cortex over an extended range of diffusion times at a b -value of $300 \text{ mm}^2/\text{s}$ (Fig. 5c). The ADC values increased almost linearly with the inverse square root of the diffusion time (Fig. 5d), with an R^2 value of 0.56 ($P < 0.0001$). This observation agrees with a previous report suggesting that $D(\omega)$ increases linearly with the square root of oscillating frequency (equivalent to the inverse square root of the diffusion time here) in the rat cortex (31). We further demonstrated the use of the DEFOG sequence to acquire diffusion tensor data (Fig. 6). The DEC maps acquired with $b = 300 \text{ s/mm}^2$ appeared noisy compared to the color maps acquired with $b = 600 \text{ s/mm}^2$, but major white matter structures could still be identified, for example, the corpus callosum, fimbria, and cerebral peduncle.

DISCUSSION

In this study, we investigated the effect of microcirculatory flow on OGSE diffusion MRI signals in the low b -value regime, as well as how to suppress this contribution to obtain accurate OGSE ADC measurements. Previous OGSE diffusion MRI studies were mostly performed on preclinical MR systems with strong gradient systems. These studies sampled the temporal diffusion spectrum over a relatively large frequency range (up to 200 Hz or higher) at moderate b -values (e.g., 700 s/mm^2) to distinguish microstructures at finer scales. Similar experiments are challenging for existing clinical MR systems. Even with the recently reported Human Connectome gradient (up to 300 mT/m), the maximum b -value that can be achieved at 200 Hz is approximately 70 s/mm^2 (estimated with two 40-ms diffusion gradient waveforms). One solution is to adopt the low b -value regime for OGSE diffusion MRI studies to sample a broad segment of the temporal diffusion spectrum.

As demonstrated by data acquired from the mouse cerebral cortex (Fig. 4), the effect of microcirculatory flow on OGSE ADC measurements is substantial at low b -values ($< 200 \text{ s/mm}^2$). Furthermore, because the degree of the microcirculatory flow effect changes with frequency, as shown in Figure 5, it may alter the shape of the estimated temporal diffusion spectrum. Both will affect how accurately we can characterize tissue microstructures based on OGSE diffusion MRI signals acquired at low b -values. The DEFOG encoding scheme suppressed the contribution from microcirculatory flow with the addition of a pulsed gradient waveform orthogonal to the oscillating gradient waveform. The advantage of the

orthogonal gradient placement is that the signal attenuation due to the pulsed gradient and the oscillating gradient can be easily separated, and the b-value of the combined gradient is the sum of the b-values of the individual gradient waveforms. This is convenient for experiments that use multiple diffusion gradient directions or strengths and that allow us to measure OGSE ADC along different directions to examine diffusion anisotropy, as shown in Figure 6. The choice of b-value for the pulsed gradient waveform (300 s/mm² in this study) was determined based on whether it could effectively suppress signals from microcirculatory flow. Because D^* was in the range of 1 to 2×10^{-2} mm²/s (21), approximately 10 times higher than the tissue water diffusion coefficient (less than 1×10^{-3} mm²/s in the mouse cortex measured in this study), signals from the model 1 type of flow would be attenuated to less than 5% of their baseline values when the b-value was set at 300 s/mm² or higher. In addition, the pulsed gradient waveform used in this study has a first-order moment (c) of approximately 4.23 rad·s/mm at $b = 300$ s/mm² (Table 1). With an estimated mouse cortical capillary flow velocity in the range of 0.5 to 2 mm/s (32), signals from the model 2 type of flow would be attenuated to less than 5%. Experimentally, a pulsed gradient waveform with a b-value greater than 300 s/mm² could effectively suppress the microcirculatory component in this and other studies (33). With these conditions, even at low b-values (50–100 s/mm² for the oscillating gradients), the ADC values measured using the DEFOG method were similar to the ADC values measured using a conventional OGSE sequence at moderate b-values. The method, however, has several limitations. First, the condition described in Eq. [2] can be reached only when the gradient modulation power spectra of both gradient waveforms have no overlap in the frequency domain. In practice, due to limited gradient waveform durations, the gradient modulation power spectra often have several sidelobes due to truncation, which may introduce a bias in the estimated ADCs. Second, the degrees at which the microcirculatory flow component is suppressed may be slightly different along different diffusion encoding directions due to differences in tissue water ADC associated with the pulsed gradient waveform, that is, D_{PGSE} in Eq. [9], along different directions. Third, the fitted ADC maps obtained using the DEFOG sequence remained noisy due to the low contrast-to-noise ratio at low b-values, as shown in Figure 2a, and the use of an additional diffusion gradient reduced the available maximum gradient strength.

One interesting finding is that, as the frequency increased, the effect of microcirculatory flow on OGSE ADC measurements decreased (Figs. 4a–b), suggesting that the effect is frequency-dependent or diffusion time-dependent. Wetscherek et al. recently reported a similar observation in the liver and pancreas using a flow-compensated pulsed gradient waveform (26). As explained in the theory section, the oscillating gradient waveform used in this study is flow-compensated; therefore, it is only sensitive to flow that passes through multiple vessel segments during the diffusion time (model 1, as defined in Le Bihan et al. (21)), whereas flow that remains in the same segment or passes through only a few segments (model 2 in Le Bihan et al. (21)) will have less attenuation (Eq. [7]) because water diffusion coefficients in the blood plasma and blood cells (2.44×10^{-3} mm²/s and 1.28×10^{-3} mm²/s, respectively) (34) are comparable to tissue water diffusion coefficients. One possible explanation is that, as the diffusion time decreases (or oscillating frequency increases), an increasing portion of the model 1 type of microcirculatory flow will become the model 2 type of flow, resulting in reduced signal attenuation at low b-values. Given an estimated

capillary flow rate of 0.5 to 2.0 mm/s (32) at an oscillating frequency of 100 Hz (equivalent diffusion time of 2.5 ms), the distance that blood travels is approximately 1.25 ~ 5 μm during the diffusion time, which is less than the average length of the blood vessel segment (~30 μm) (28). It is conceivable that, at ultrashort diffusion times (e.g., < 1 ms), most capillary flow will not travel through one vessel segment, and there should be no apparent microcirculatory flow effect from model 1. Further investigation is needed to assess the diffusion time-dependent effect of microcirculatory flow because it may provide additional information about capillary geometry and flow velocity. Given multiple b-value measurements, it is possible to examine the multicompartiment microcirculatory flow and its diffusion time-dependency with proper models and numerical simulations (21–25).

CONCLUSION

In summary, we found that the effect of microcirculatory flow was present in OGSE diffusion MRI measurements acquired at low b-values and might alter the shape of temporal diffusion spectrum. We proposed a new diffusion preparation method with orthogonally oriented pulsed or oscillating gradients to suppress this effect. With the new method, accurate OGSE-ADC measurements can be obtained in the low b-value regime, which is important for clinical applications of the OGSE technique.

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FIG. 1.

Pulsed and oscillating gradients in two schemes of combinations and their power spectra. In each column, the top panel shows the basic timing diagram of the RF pulses and gradient waveforms. (a–b) Combination of a pulsed and an oscillating gradient (a) or two oscillating gradients (b) in the same direction. In this case, there are usually mismatches (indicated by the red arrows) between the modulation power spectra of the combined gradients ($|F(\omega)|^2$) and the sum of individual gradient modulation power spectra ($|F_1(\omega)|^2$ and $|F_2(\omega)|^2$). (c–d) The dual-frequency orthogonal gradients sequence with orthogonally placed pulsed and oscillating gradients (c) or two oscillating gradients (d). In these cases, the $|F(\omega)|^2$ of the combined gradient are equal to the sum of each gradient waveform's modulation power spectra. RF, radio frequency.

FIG. 2.

Comparisons of PGSE, OGSE, and DEFOG ADC maps. (a) Representative PGSE, OGSE (100 Hz), and DEFOG ADC maps (100 Hz) of an adult mouse brain, acquired with b-values of 200 s/mm² (top row) and 500 s/mm² (bottom row). For the DEFOG sequence, the b-value of the pulse gradient waveform was 300 s/mm². The unit of the grayscale bar is 10⁻³ mm²/s. (b) Corresponding T₂WT and FA images. Regions of interest for the cortex (yellow) and cerebral peduncle (orange) were defined in the FA image. ADC, apparent diffusion coefficient; DEFOG, dual-frequency orthogonal gradients; FA, fractional anisotropy; OGSE, oscillating gradient spin echo; PGSE, pulsed gradient spin echo; T₂WT, T₂-weighted.

FIG. 3.

Histograms of the six apparent diffusion coefficient maps shown in Figure 2a. The unit of the horizontal axis is $10^{-3} \text{ mm}^2/\text{s}$. DEFOG, dual-frequency orthogonal gradients; OGSE, oscillating gradient spin echo; PGSE, pulsed gradient spin echo.

FIG. 4.

Plots of ADC values in the mouse cortex (a) and cerebral peduncle (b) acquired using conventional PGSE (solid lines), oscillating gradient spin echo (100 Hz and 200 Hz, solid lines) and DEFOG sequences (100 Hz-DEFOG and 200 Hz-DEFOG, dashed lines) over a b-value range of 50 to 700 s/mm². The data were presented as mean $\pm$ standard error of the mean ($n = 5$). The values were obtained from the regions of interest defined in the fractional anisotropy images. *** $P < 0.001$; ** $P < 0.01$ from the Bonferroni posthoc tests. ADC, apparent diffusion coefficient; DEFOG, dual-frequency orthogonal gradients; PGSE, pulsed gradient spin echo.

FIG. 5.

(a–b) Changes in conventional PGSE/OGSE and DEFOG ADC values at $b = 100 \text{ s/mm}^2$ and $b = 150 \text{ s/mm}^2$. The dashed lines indicate the slopes of the linear regression of the ADC b-values against the diffusion times. The error bars indicate standard deviations among subjects ($n = 5$). (c–d) ADC measured at $b = 200 \text{ s/mm}^2$ plotted against the diffusion times (c) and the inverse square root of the diffusion times (d). ** $P < 0.01$ from the Bonferroni posthoc tests. ADC, apparent diffusion coefficient; DEFOG, dual-frequency orthogonal gradients; OGSE, oscillating gradient spin echo; PGSE, pulsed gradient spin echo.

FIG. 6.

Diffusion tensor reconstruction using PGSE, OGSE, and DEFOG sequences at several b-values. Both fractional anisotropy and direction-encoded color-maps are shown here. red = left/right; green=dorsal/ventral; and blue=rostral/caudal. ADC, apparent diffusion coefficient; cc, corpus callosum; cp, cerebral peduncle; DEFOG, dual-frequency orthogonal gradients; fi, fimbria; OGSE, oscillating gradient spin echo; opt, optical tract; PGSE, pulsed gradient spin echo.

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Table 1

The values of key components in Eq. [9] related to the pulsed gradient waveform $G_2(\theta)$ ($\delta = 10$ ms and $\tau = 13.82$ ms) with different b_2 values. To calculate these values, we assume that $v = 1$ mm/s (32), $D_{PGSE} = 0.6 \times 10^{-3}$ mm²/s (ADC values measured in the cortex from this study), and $D^*_{PGSE} = 0.01$ mm²/s

b_2 (s/mm ²)	G_2 (mT/m)	$e^{-b_2 \cdot D^*_{PGSE}}$	c_2 (rad·s/mm)	$ \sin(c_2)/c_2 $	$e^{-b_2 \cdot D_{PGSE}}$
50	27.1	0.61	1.73	0.14	0.97
100	38.3	0.37	2.44	0.13	0.94
200	54.2	0.14	3.46	9.1×10^{-2}	0.89
300	66.4	5.0×10^{-2}	4.23	5.0×10^{-2}	0.84
400	76.7	1.8×10^{-2}	4.89	2.2×10^{-2}	0.79
500	85.7	6.7×10^{-3}	5.47	5.8×10^{-2}	0.74

ADC, apparent diffusion coefficient; D^* , pseudo-diffusion coefficient; PGSE, pulsed gradient spin echo.

Table 2

ADC measured in an agarose gel (4% by weight) phantom with conventional PGSE and OGSE sequences and the DEFOG-OGSE sequence with b-values ranging from 50 to 700 s/mm². The measurements were repeated three times. Data are presented as mean ± standard deviation in the unit of 10⁻³ mm²/s

b-value (s/mm ²)	50	100	150	200	300	400	500	600	700
PGSE	2.21 ± 0.03	2.21 ± 0.03	2.20 ± 0.03	2.20 ± 0.03	2.20 ± 0.03	2.20 ± 0.03	2.19 ± 0.03	2.19 ± 0.03	2.19 ± 0.03
OGSE 100 Hz	2.20 ± 0.02	2.19 ± 0.02	2.17 ± 0.02	2.17 ± 0.02	2.18 ± 0.02	2.17 ± 0.02	2.17 ± 0.02	2.17 ± 0.02	2.17 ± 0.02
DEFOG 100 Hz	2.21 ± 0.02	2.19 ± 0.01	2.18 ± 0.01	2.18 ± 0.01	2.18 ± 0.01	2.18 ± 0.01	2.18 ± 0.01	2.18 ± 0.01	2.18 ± 0.01
OGSE 200 Hz	2.21 ± 0.02	2.20 ± 0.02	2.20 ± 0.02	2.19 ± 0.02	2.19 ± 0.02	2.18 ± 0.02	2.18 ± 0.02	2.18 ± 0.02	2.18 ± 0.02
DEFOG 200 Hz	2.22 ± 0.02	2.22 ± 0.02	2.21 ± 0.02	2.20 ± 0.02	2.20 ± 0.02	2.20 ± 0.02	2.20 ± 0.02	2.20 ± 0.02	2.20 ± 0.02

ADC, apparent diffusion coefficient; DEFOG, dual-frequency orthogonal gradients; OGSE, oscillating gradient spin echo; PGSE, pulsed gradient spin echo.

Table 3

CV of the ADCs in the cortical region in five mice measured using the OGSE sequence and the DEFOG sequence at an oscillating frequency of 100 Hz

b-value (s/mm ²)	100 Hz		200 Hz	
	OGSE	DEFOG	OGSE	DEFOG
50	0.083	0.072	0.090	0.039
100	0.114	0.076	0.056	0.045
150	0.073	0.075	0.049	0.050
200	0.046	0.070	0.057	0.045
300	0.062	0.063	0.038	0.046
400	0.057	0.065		
500	0.053	0.058		
600	0.055	0.070		
700	0.054	0.080		

ADC, apparent diffusion coefficient; CV, coefficient of variation; DEFOG, dual-frequency orthogonal gradients; OGSE, oscillating gradient spin echo.