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Probing Mouse Brain Microstructure Using Oscillating Gradient Diffusion Magnetic Resonance Imaging

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Abstract

High resolution diffusion tensor images of the mouse brain were acquired using the pulsed gradient spin echo (PGSE) sequence and the oscillating gradient spin echo (OGSE) sequence. The OGSE tensor images demonstrated frequency-dependent changes in diffusion measurements, including apparent diffusion coefficient (ADC) and fractional anisotropy (FA), in major brain structures. Maps of the rate of change in ADC with oscillating gradient frequency revealed novel tissue contrast in the mouse hippocampus, cerebellum, and cerebral cortex. The observed frequency-dependent contrasts resembled neuronal soma-specific Nissl staining and nuclei-specific DAPI staining in the mouse brain, which suggests that the contrasts might be related to key features of cytoarchitecture in the brain. In the mouse cuprizone model, OGSE-based diffusion MRI revealed significantly higher frequency-dependence of perpendicular diffusivity ($\lambda_{\perp}$) in the demyelinated caudal corpus callosum at 4 weeks after cuprizone treatment, compared to control mice and mice at 6 weeks after cuprizone treatment. The elevated frequency-dependence of $\lambda_{\perp}$ coincided with the infiltration of activated microglia/macrophages and disruption of axons during acute demyelination in the caudal corpus callosum. The results demonstrate the potential of OGSE-based diffusion MRI for providing tissue contrasts complimentary to conventional PGSE-based diffusion MRI.

Keywords

oscillating gradient; diffusion tensor imaging; mouse brain; cerebellum; hippocampus; microstructure

INTRODUCTION

Diffusion magnetic resonance imaging (dMRI) is a versatile technique for studying tissue microstructure in the central nervous system (CNS) and its pathology, such as tumor, stroke,

and multiple sclerosis (1-5). In the presence of structural barriers such as cell membranes and organelles, diffusion of water molecules in the CNS is restricted. Because of the restrictive effects of structural barriers, MRI measurable properties of water diffusion such as apparent diffusion coefficient (ADC) and diffusion anisotropy can provide unique information about tissue structure at the micrometer scale, far finer than the size of individual image pixels, which is mostly in the millimeter to sub-millimeter range (6). Advanced dMRI techniques, such as diffusion tensor imaging (DTI) (7,8) and q-space imaging (9), have been developed to study various aspects of microscopic tissue composition and organization.

For restricted diffusion, there are several imaging parameters that influence the sensitivity of a dMRI experiment. One key parameter that determines the sensitivity of dMRI to microstructures at different spatial dimensions is the diffusion time (t_D), which is the time period during which the diffusion of water molecules within their microenvironment is measured. With increasing diffusion time, the possibility of detecting the restrictive effects of structural barriers over larger dimensions (but still much smaller than the dimension of a pixel) increases. For example, the one-dimensional root-mean-square displacement of water molecules undergoing diffusion in a free medium at body temperature is about 11 μm in 20 milliseconds, or 22 μm in 80 milliseconds. In biological tissues, there are numerous potential structural barriers to water diffusion and the sizes of these barriers vary over a large range, from cell organelles to cell membranes to extracellular structures (10). With longer diffusion times it is likely for water molecules to encounter more barriers or barriers over longer spatial scales, thereby leading to less signal attenuation in a dMRI experiment than in similar experiments with the same diffusion-weighting (b -value) but shorter diffusion time. This diffusion time-dependent behavior has been observed in packed cells, skeletal muscle, and brain (11-14). Because signal attenuation in dMRI reflects the combined effects of barriers at different spatial scales within a voxel, by acquiring dMRI measurements at different diffusion times, it may be possible to separate or highlight structures with different spatial dimensions. The ability to do this can potentially provide tissue contrasts that are sensitive to the spatial heterogeneity of cell and tissue microstructures in the CNS.

It is, however, not straightforward to implement dMRI experiments with short diffusion times that are needed to generate such contrasts in the CNS by distinguishing barriers at microscopic spatial scales. Most contemporary dMRI experiments are based on the pulsed gradient spin echo (PGSE) sequence (15), which employs a pair of identical diffusion sensitizing gradient pulses (G). Shortening the diffusion time in the PGSE sequence requires increasing the magnitude of the diffusion gradient to maintain its sensitivity. This requirement makes extremely short diffusion times difficult to implement in PGSE-based dMRI experiments, due to limitations of current gradient hardware.

One alternative is to use the so-called oscillating gradient spin echo (OGSE) sequence (16-18), which replaces the pulsed gradients in the PGSE sequence with oscillating gradient waveforms. The OGSE sequence accommodates short diffusion times better than the conventional PGSE sequence, and more importantly, provides a unique way to measure water diffusion. Similar to DTI based on the PGSE sequence, dMRI measurements obtained using the OGSE sequence at different oscillation frequencies can be fitted into a series of tensors as a function of oscillation frequency or diffusion time, $D(f)$. Following the work of Stepisnik (19,20), the diffusion tensor can be constructed as the Fourier transform of the spin velocity autocorrelation function, i.e., a function of frequency. Given a diffusion gradient waveform $G(t)$, the signal attenuation due to diffusion in this case follows

$$S = S_0 \exp \left(- \int_{-\infty}^{\infty} \mathbf{F}(f) \mathbf{D}(f) \mathbf{F}(f) df \right) \quad [1]$$

where

$$F(f) = \int_0^\infty dt \exp(i2\pi ft) \int_0^t d\tau \gamma G(\tau) \quad [2]$$

In equation 2, γ is the gyromagnetic ratio. It is clear that the frequency characteristic of $F(f)$ has a direct impact on the signal attenuation, and that it is possible to sample $D(f)$ using specially designed $G(t)$. Because the $F(f)$ of the gradient pulses in the PGSE sequence localizes around the zero frequency (21), the results of conventional PGSE-based DTI reflect mainly the zero frequency component of $D(f)$. Several recent reports have introduced modified oscillating gradient waveforms that allow short diffusion times and selective sampling of the diffusion spectrum (21,22). Applications of these sequences in measuring structural properties in phantoms as well as rodent models of stroke and brain tumor have demonstrated the potential of oscillating gradient dMRI (23,24).

In this study, diffusion tensor images of the mouse brain using oscillating gradient waveforms were acquired. We chose the mouse brain because it is widely used in biomedical research and numerous mouse models have been developed to study the molecular mechanisms and treatment of human diseases. The aim is to investigate whether oscillating gradient dMRI can generate additional tissue contrasts for studying mouse brain anatomy. Full tensor data were acquired in order to investigate the effect of diffusion time both on mean diffusivity and diffusion anisotropy measures. We also acquired images from mice with cuprizone-induced demyelinating lesions (25) to investigate if oscillating gradient dMRI could provide new contrasts sensitive to white matter pathology.

MATERIALS AND METHODS

Animals and Specimen Preparation

All experimental procedures were approved by the Animal Use and Care Committee at the Johns Hopkins University School of Medicine. Adult C57BL/6 mice (two month old, female) were separated into two cuprizone-treated groups and a control group. Mice in the two cuprizone-treated groups ($n=5$ for each group) were placed on a 0.2% cuprizone-enriched diet, and sacrificed at 4 and 6 weeks after the start of the diet, respectively. Mice in the control group ($n=5$) were kept on a normal diet and sacrificed together with the 6 week cuprizone-treated group. A separate group of normal adult C57BL/6 mice (two month old, female, $n=4$) were used for high resolution three dimensional (3D) imaging. The control and cuprizone-treated mice were fixed by trans-cardiac perfusion of 4% PFA in phosphate buffered saline (PBS) for *ex vivo* MRI and histology. After fixation, the mouse heads were removed and immersed in 4% PFA in PBS for 16 hours at 4°C before being transferred to PBS with 2 mM gadopentetate dimeglumine (Magnevist, Berlex Imaging, Wayne, NJ, USA). During imaging, the specimens were placed into custom-built MR-compatible tubes. The tubes were filled with Fomblin (Fomblin Profludropolyether, Ausimont, Thorofare, New Jersey, USA), which is an MR-invisible liquid for susceptibility matching and also prevents dehydration (26,27).

Image Acquisition

Since the main pathology in the cuprizone mouse model is located in the caudal corpus callosum, two dimensional (2D) sagittal diffusion-weighted images were acquired from the control and cuprizone-treated mouse brain specimens. Imaging was performed on a vertical bore 11.7 Tesla NMR spectrometer (Bruker Biospin, Billerica, MA, USA) equipped with a Micro 2.5 gradient system (maximum gradient strength of 1000 mT/m). A 15 mm diameter volume coil was used for radio frequency transmission and reception. During imaging, the

temperature of the specimens was maintained at 37°C via the spectrometer's temperature control system. Sagittal T₂-weighted images of the mouse brain were acquired using the fast spin echo sequence with an echo time (TE) of 40 ms, repetition time (TR) of 2000 ms, echo train length of 4, 8 signal averages, field of view (FOV) of 20 mm × 10 mm, matrix size of 256 × 128, and 3 slices with a slice thickness of 1 mm (no gap). The locations of the slices were carefully selected to ensure that the mid-sagittal plane of the brain was imaged. Four sets of coregistered diffusion-weighted images were acquired with the same FOV, matrix size, and slice thickness as the T₂-weighted images. The first set of diffusion-weighted images was acquired using a standard pulsed gradient spin echo (PGSE) sequence with a TE of 50 ms and a TR of 1000 ms, while the other three sets of diffusion-weighted images were acquired using the OGSE-c1 waveform as described in (21) with a TE of 50 ms and a TR of 1000 ms. The OGSE-c1 waveform consisted of two cosine-modulated gradients of frequency f with the initial and last half cosine lobes replaced by half-sine pulses of frequency $2f$ (Fig. 1, B-D) (21), whose areas equal the areas of the half cosine lobes they replace. Each set of images contained two images with minimal diffusion weighting (b_0 images, b -value = 40 s/mm² and 28 s/mm² for the PGSE and OGSE experiments, respectively) and six images with heavy diffusion weighting (b_1 images, b -value ~ 700 s/mm²). The directions of the six b_1 images were: [1,1,0], [1,0,1], [0,1,1], [-1,1,0], [1,0,-1], and [0,-1,1]. The b -value and effective diffusion time (t_D) for the PGSE sequence were

defined as $b_{PGSE} = \gamma^2 G^2 \delta^2 \left(\Delta - \frac{\delta}{3} \right)$ and $t_D = \Delta - \frac{\delta}{3}$, whereas the b -value and effective diffusion

time for the OGSE sequence were defined as $b_{OGSE} = \frac{\gamma^2 G^2 N}{4\pi^2 f^3} \left(1 - \frac{1}{8N} \right)$, and $t_D = \frac{1}{4f}$, according to (28), with G being the strength of the diffusion gradient, δ and Δ the duration and separation of the diffusion gradient pulses in the PGSE sequence, f the frequency of the oscillating gradients, and N the number of periods in each waveform. The total acquisition time was ~9 h for each specimen. Table 1 shows the details of the parameters for the four sets of diffusion-weighted images. The signal-to-noise ratios measured in the b_0 images were ~78 and ~83 for the PGSE and OGSE sequences respectively. Figure 1 shows the gradient waveforms and the spectra of their corresponding $F(f)$ (Fig. 1E). The ripples in the spectra are due to truncation of the gradient waveform, and, in the case of the OGSE sequence, also due to modifications made to the cosine waveform (21). While the spectrum of the 50 Hz oscillating gradient has major overlap with the spectrum of the PGSE sequence, the peaks of the spectra of the 100 Hz and 150 Hz gradient waveforms are well separated from the peak of the spectrum of the PGSE sequence. Because the spectrum of the PGSE sequence is localized around 0 Hz, dMRI measurements obtained using the PGSE sequence are referred to as measured at 0 Hz in comparison with measurements obtained using the OGSE sequence.

For 3D acquisition, the normal mouse brains were dissected from the skull. High resolution images of these brains were acquired on the same spectrometer using a 3D diffusion-weighted gradient and spin echo (GRASE) sequence (29) modified with oscillating diffusion gradient waveforms. Images were acquired with an FOV of 11.5 mm × 7.2 mm × 15.1 mm, matrix size of 128 × 80 × 168, 4 signal averages, and a TE/TR of 55/700 ms. The total acquisition time was ~28 h for each brain. The diffusion parameters used for the 3D acquisition were identical to those used for the 2D acquisitions as shown in Table 1. The acquired images had a native spatial resolution of 0.09 mm × 0.09 mm × 0.09 mm. The signal-to-noise ratios measured in the 3D b_0 images were ~70 and ~68 for the PGSE and OGSE sequences, respectively.

Image Processing and Statistical Analysis

For both 2D and 3D images, diffusion tensor fitting was performed using the Log-linear fitting function in DtiStudio (30). The primary, secondary, and tertiary eigenvalues (λ_1 , λ_2 , λ_3 respectively) and corresponding eigenvectors were calculated. Fractional anisotropy (FA) (7), apparent diffusion coefficient (ADC), parallel diffusivity ($\lambda_{\parallel} = \lambda_1$), and perpendicular diffusivity ($\lambda_{\perp} = (\lambda_2 + \lambda_3)/2$) were also calculated. Direction-encoded colormap (DEC) images were generated by combining the images of the primary eigenvector and FA into RGB images. To compute the frequency-dependence of diffusion measurements in the brain, the estimated tensors at different frequencies, $\mathbf{D}(f)$, were fitted to a linear model. First, from the estimated tensors, the ADCs at each frequency were calculated. Let v_1 , v_2 and v_3 denote the eigenvectors of the diffusion tensor measured using the PGSE sequence, then the diffusivities at each frequency along the three orthogonal axes defined by v_1 , v_2 and v_3 were calculated as $v_1^T \mathbf{D}(f) v_1$, $v_2^T \mathbf{D}(f) v_2$ and $v_3^T \mathbf{D}(f) v_3$, respectively. This procedure was introduced in case the three principal orthogonal axes changed with frequency. Linear least squares fittings of ADC and each of the estimated diffusivities with the oscillation frequency were then performed using IDL (ITT Visual Information Solutions, Boulder, AZ, USA). The results were maps representing the rates of change in ADC and diffusivities with the frequency of oscillating gradients. The average rate of changes in diffusivities along v_2 and v_3 was computed to generate a map representing the degree of frequency-dependence of perpendicular diffusivity. Regions of interest (ROIs) within the brain were manually placed using ROEditor (<http://www.mristudio.org>) to obtain the mean FA, ADC, parallel and perpendicular diffusivities, and other OGSE measurements in each ROI. Wilcoxon rank-sum tests were used for group comparison since the FA and diffusivity measurements were not normally distributed. All statistical tests were performed using Sigma Plot (SigmaPlot 10.0, Systat Software, San Jose, CA, USA).

Immunohistochemistry

Mouse brains were stained for nuclei (DAPI), axon markers (SMI-31 for phosphorylated neurofilament), and neuroinflammatory markers (iba-1 for activated microglia). Briefly, 7 micron paraffin-embedded sections were mounted on to SuperfrostPlus slides, dried, and heated at 60°C for 30 minutes. Wax was removed from sections with S3-Histo™ (xylene substitute), immersed in 100% ethanol, and hydrated in 95, 70, 50, 30% ethanol and finally in water. Antigen retrieval was performed by boiling sections in 50mM Tris-HCl, pH 9.5 for 15 minutes followed by quick cooling. Slides were immersed in 3% H₂O₂ (which reduces autofluorescence) for 30 minutes and washed. Nonspecific antibody interactions were blocked by applying 5% goat serum in PBS+0.1% Tween-20 to each sample for 30 minutes. Immuno-labeling with primary antibodies was performed overnight at 4°C: rabbit iba-1 (Wako, USA): 1:1000, mouse anti-phosphorylated neurofilament, clone SMI31: 1:2500. Slides were washed and secondary antibody applied for 45 minutes (goat anti-rabbit AlexaFluor594 and anti-mouse AlexaFluor 488 (both from Molecular Probes/Invitrogen, both used at 1:250 in blocking solution)); blocking and antibody steps employed CoverWell® incubation chambers (RPI, Inc.) and covered slides were placed in a humid chamber for the duration of the incubation. Sections were then washed in PBS+0.1% Tween-20 twice, with PBS without Tween-20 three times, and with distilled water (dH₂O) three times to remove all traces of Tween-20. To reduce autofluorescence inherent in paraffin-embedded sections, slides were first immersed in 70% ethanol for 5 minutes, then in 0.5% (w/v) Sudan Black B in 70% ethanol for 1.5 minutes, and washed quickly and repeatedly with H₂O (too long an exposure to Sudan Black will cause fading of specific staining) (31). To make Sudan Black solution, stain was added to 70% ethanol and mixed overnight, clarified by centrifugation at 1900g for 10 minutes, and filtered through Whatman paper just prior to use. Slides were then mounted with anti-fade mounting media, (Gel-Mount (EMS)) supplemented with 0.5µg/ml 4',6-diamidino-2-phenylindole (Molecular

Probes/Invitrogen; DAPI, blue nuclear stain) and sealed with clear nail polish to prevent formation of air bubbles. Slides were stored at 4°C until viewing and protected from light.

RESULTS

ADC, FA, and colormap images generated from *ex vivo* mouse brain tensor data acquired using both PGSE and OGSE sequences (at 50, 100, and 150 Hz) are shown in Figure 2. Mean ADC, FA and diffusivity values for the normal mouse brains, from representative ROIs in grey matter regions and major white matter tracts are plotted in Figure 3. ADC measurements in most brain regions increased with oscillation frequency. FA values of several white matter tracts were found to decrease slightly with increasing oscillation frequency (Fig. 3B). The decreases in FA values in these white matter tracts were associated with relatively stronger frequency-dependent increase in $\lambda_{\perp}$ compared to $\lambda_{\parallel}$ (Fig. 3C-D). No apparent change in the primary orientation of water diffusion in the brain with changing oscillation frequency was found in the colormap images (Fig. 2 DEC maps).

Specific regions in the mouse hippocampus and cerebellum showed interesting tissue contrast enhancement with oscillating-gradient dMRI. Figure 4A compares coronal and sagittal ADC images of the mouse hippocampus to Nissl stained histology from the Paxinos' mouse brain atlas (32). At frequencies of 100 to 150 Hz, ADC values in two regions that approximate the granule cell layer of the dentate gyrus (GrDG) and the pyramidal cell layer of the hippocampus (Py) were enhanced. In the ADC maps acquired using the PGSE sequence, only the region that approximates the Py can be distinguished from the surrounding tissue, with the mean ADC in the Py being slightly higher than the mean ADC in the GrDG. As the oscillation frequency increased, the mean ADCs of both Py and GrDG increased significantly ($p < 0.005$) and their ADC values became less distinguishable (Fig. 4C). The drastic frequency-dependent increase in ADCs was specific to the Py and GrDG regions, whereas other hippocampal regions (e.g., CA1 in Fig. 4C) showed only a slight increase in ADC with frequency. Figure 4B shows a comparison of the mid-sagittal cerebellum in the ADC images to T_2 -weighted MR images and Nissl stained histology. Again, at 100 to 150 Hz, the contrast between the molecular layer of the cerebellar cortex (CBML) and the underlying granule cell layer (CBGr) was enhanced compared to the PGSE result. The cerebellar cortex has a highly organized cytoarchitecture, with an innermost granule cell layer consisting primarily of the cell bodies of densely-packed granule cells, whose axons project into the outermost molecular layer. While the mean ADC in the CBML exhibited a slight increase with increasing oscillation frequency, the mean ADC in the CBGr increased dramatically and became significantly ($p < 0.001$) different from the mean ADC of the CBML at frequencies of 100 and 150 Hz (Fig. 4C and Table 2). Nuclei-specific DAPI and axon-specific SMI-31 staining of sections through the cerebellum and hippocampus (Fig. 5) showed that the CBGr, GrDG, and Py were regions characterized by densely packed neurons with relatively large nuclei and thin cytoplasm.

By fitting the diffusion tensor data acquired at different frequencies to a linear model, maps of the rate of change in ADC with respect to gradient oscillation frequency were obtained. Regions that showed more rapid frequency-dependent increase in measured ADC than other regions appeared hyperintense in the fitted maps (Fig. 6). The mean ($\pm$ standard deviation) fitted slopes of ADC versus frequency in different grey matter regions in the normal mouse brains obtained by the linear fitting procedure are listed in Table 3. As expected, the GrDG, Py and CBGr regions were highlighted. Other regions that also showed apparent enhancement include a layer in the olfactory bulb, a layer in the piriform cortex, and interestingly, a layer in the cerebral cortex. Compared to Nissl stained histology, areas of hyperintensity in the fitted maps were located mostly in regions with densely packed neurons (strong Nissl staining) (Fig. 6).

Figure 7 shows *ex vivo* images of the normal corpus callosum in control mice and the demyelinated caudal corpus callosum in cuprizone-treated mice after 4 weeks and 6 weeks of treatment. In both PGSE and OGSE experiments, the FA values in the caudal corpus callosum were lower in the cuprizone-treated mice than in the control mice. As frequency increased, FA decreased and perpendicular diffusivity increased (Fig. 7B & 7C). In the PGSE results, no significant difference in perpendicular diffusivities between the 4 week and 6 week time points following cuprizone-treatment was found ($\lambda_{\perp}$ maps in Fig. 7A). However, there were significant differences ($p < 0.002$) in the $\lambda_{\perp}$ measured using oscillating gradients at 100 and 150 Hz between the two time points. Furthermore, the rate of frequency-dependent increase in $\lambda_{\perp}$ in the caudal corpus callosum was found to be highest at the 4 week time point, and at this point the rate of change was significantly ($p < 0.005$) higher compared to the control (0 week) as well as the 6 week time points (Fig. 7D). Immunolabeled histological sections (Fig. 8) revealed swelling of the corpus callosum and reduced SMI-31 staining at the lesion site at the 4 week time point, suggesting disrupted axoplasmic flow. At this point, a large number of iba-1 positive cells could be seen in the corpus callosum, which likely represent both activated microglia and macrophages. In the control mice, the axons in the corpus callosum were well organized with only a few iba-1 positive cells among them. At 6 weeks after cuprizone treatment, the size of the corpus callosum also appeared relatively normal and the number of iba-1 positive cells was greatly reduced, but SMI-31 labeled axons still appeared disrupted.

DISCUSSION

This study investigated the application of oscillating gradient dMRI to study tissue microstructure and white matter pathology in the mouse brain. We found new frequency-dependent tissue contrasts in the mouse brain and also demonstrated the sensitivity of this method to microstructural changes associated with white matter pathology in the cuprizone mouse model. The results indicate that this technique can potentially be used to explore multi-scale restrictive effects of various tissue components and reveal additional structural information in the CNS.

There have been several reports on using the oscillating gradient dMRI technique to study systems of restricted diffusion (16,21-23,33,34). Frequency or diffusion time dependence of ADC measurements have been demonstrated in phantoms (21,23). ADC measurements in the rat cortex and sub-cortical grey matter have been shown to increase with frequency (24). With the vast heterogeneity in tissue microstructural properties and organization within the brain, sampling the diffusion spectrum over different frequency domains can provide complementary tissue contrasts to distinguish microscopic structures in regions that otherwise appear homogeneous in conventional PGSE-based dMRI results. In the present study, high-resolution 3D diffusion tensor images acquired with relatively high frequency oscillating gradients revealed novel tissue contrasts in the mouse brain. The ADCs measured in several grey and white matter structures increased with the frequency of the oscillating gradients. We discovered that certain structures in the cerebellum (the CBGr layer) and the hippocampal formation (the GrDG and Py layers) were highlighted by significant frequency-dependent ADC increase. From maps representing the rate of change in ADC versus frequency, regions within the cerebral cortex, olfactory bulb, and the piriform cortex also showed enhancements. These findings suggest that oscillating gradient dMRI can generate unique tissue contrasts not available from conventional PGSE based experiments.

Our knowledge on the potential mechanisms of the tissue contrasts reported here remains limited. Using phantoms consisting of impermeable microspheres ranging from 1 μm to 400 μm in diameter, Parsons *et al.* (21) reported that, as the oscillation frequency increased from 0 to 200 Hz, ADCs measured in the phantoms with large microspheres increased faster with

frequency than the phantoms with small microspheres. Another report by Xu *et al.* (28) used simulations to study the effects of the ratio of nuclear volume to cellular volume, or nuclear volume fraction, on ADC measurements. They demonstrated that while the PGSE results showed no change in ADC measurements with this ratio, ADCs measured using the OGSE sequence increased with this ratio. The simulation results also showed that the rate of change in ADC with frequency increased with the nuclear volume fraction. These reports and the correlations between the highlighted regions in the fitted ADC versus frequency maps and staining intensity in the soma-specific Nissl /nuclei-specific DAPI stained sections suggest that the tissue contrasts observed in this study may be related to certain cytoarchitectural features in these regions. These highlighted regions all contain densely packed neurons, which resemble the model of close-packed spherical cells constructed by Xu *et al.* for their simulations (28). Two of the highlighted regions with high rates of increase in ADC with frequency, the CBGr and GrDG layers, contain a large population of densely packed granule cells, which are among the smallest neurons in the brain and have a thin cytoplasm (35). Another highlighted region, the Py region in the hippocampus, consists of densely packed pyramidal cells, which have relatively larger soma and more cytoplasm than the granule cells. Quantitative data on the cellular and nuclear morphology of these cells are rather limited. The diameters of the granule and pyramidal cells (5-6 μm for the granule cells in the cerebellum, 10-18 μm for the granule cells in the dentate gyrus, and approximately 20 μm for the pyramidal cells in the hippocampus) (35-37, <http://neurolex.org>) are comparable to the one-dimensional root-mean-square displacements of water molecules within the range of diffusion times used in this study (3-10 μm within 1.67-15 ms at 37°C). The granule cells in the CBGr have the thinnest cytoplasm, with only 0.03 - 0.5 μm distance between the nuclear surface and cell membrane (38). The nuclei of the granule cells in the GrDG have an average diameter of 5.66 μm (39), and the diameters of the nuclei of the pyramidal cells in the hippocampus are approximately 6.5 - 7.4 μm (40). Assuming spherical shaped cells and nuclei, the nuclear volume fractions of these cells are approximately 0.3 - 0.7 for the granule cells in the CBGr, 0.03 - 0.18 for the granule cells in the GrDG, and 0.03 - 0.05 for the pyramidal cells in the Py region. In comparison, the granule cells in the CBGr had the highest rate of frequency-dependent increase in ADCs, followed by the granule cells in the GrDG, and then the pyramidal cells in the Py (Table 3). These results suggest that the measured rate of ADC changes with frequency as well as the associated tissue contrasts may be correlated with the nuclear volume fractions in these densely packed neuronal regions, as described in (28). Further investigations with simulations and more detailed cytoarchitectural analyses are necessary to identify other factors that may contribute to the tissue contrast and clarify the mechanisms that govern contrast generation in oscillating gradient dMRI.

Using the tensor model of diffusion, the frequency dependent behaviors of $\lambda_{\parallel}$, $\lambda_{\perp}$, and FA in the mouse brain white matter were studied. ADC and diffusivities measured in several white matter structures increased with frequency. Because $\lambda_{\perp}$ increased more rapidly than $\lambda_{\parallel}$ in the white matter structures studied here, the corresponding FA values decreased with frequency. We did not observe any apparent frequency-dependent change in the primary orientation of anisotropy in these tracts or any grey matter regions that have a predominant diffusion orientation in the PGSE results, although such changes may be possible at higher frequencies than achieved in this study. We further examined the sensitivity of oscillating gradient dMRI to white matter pathology in the well characterized mouse cuprizone model. In this model, profound demyelination (90%) can be consistently observed in the caudal corpus callosum at 4-5 weeks after the start of a cuprizone diet (41). The application of oscillating gradient DTI in the cuprizone model showed significantly increased $\lambda_{\perp}$ in the caudal corpus callosum at the 4 and 6 week time points. The increases in $\lambda_{\perp}$ at the 4 and 6 week time points measured using the PGSE sequence have been attributed to demyelination in this model (42,43). These results suggest that $\lambda_{\perp}$ measured using the OGSE sequence at

the frequencies used in this study was also sensitive to demyelination. The significant increases in OGSE based $\lambda_{\perp}$ at 100 and 150 Hz and frequency-dependence of $\lambda_{\perp}$ in the caudal corpus callosum at the 4 week time point compared to the 6 week time point is interesting, because no significant difference was found between the PGSE-based $\lambda_{\perp}$ between the two time points in this study. Swelling of the caudal corpus callosum due to infiltration of immune cells in the cuprizone model has been reported before (44), and can also be appreciated in our immuno-stained sections in Fig. 8. These pathological changes might be the underlying causes of the increase in frequency-dependence of $\lambda_{\perp}$ since the size of the microglia, as barriers to water diffusion, is much larger than the diameter of axons ($< 1 \mu\text{m}$) and can result in an increase in the rate of frequency-dependent changes in ADC. These findings demonstrate the potential of oscillating gradient DTI in characterizing white matter pathology, and suggest that changes in OGSE based diffusion measurements may be indicative of microscopic changes induced by white matter pathology, occurring over spatial scales that conventional PGSE-based DTI may not be sensitive to. It is, however, necessary to perform additional studies with detailed histopathological analyses to evaluate the sensitivity of OGSE based dMRI to white matter pathology with respect to conventional PGSE based dMRI.

Based on our current understanding of the origins of diffusion MR signals, more detailed analysis of the frequency-dependent contrast changes, for instance estimating the relative contributions of intra- and extra-cellular compartments, is difficult. Advanced mathematical models have been proposed to study the time-dependence of diffusion in restricted systems with known geometries (11,12,45). For the range of b -values used in our study, it has been shown that the signal decay in the PGSE-based diffusion MR experiments is dominated by the fast diffusing component, which has been suggested to originate from the extracellular space (46). However, further studies are necessary to investigate the exact correlates of the restriction effects responsible for frequency-dependent dMRI contrast changes observed with the OGSE technique. Further optimization of the imaging protocol requires detailed understanding of the underlying contrast generation mechanism, in order to determine the range of diffusion times and diffusion-weighting (b -values) to obtain sensitivity to specific structures. It is necessary to note that the images presented here were acquired from postmortem brain specimens perfusion fixed with 4% PFA. Several studies have shown that PFA fixation can result in significant changes in tissue microstructural properties, such as increased apparent restriction size and membrane water exchange (47,48). As a result, the frequency dependent changes in ADC measured in live animal brains may differ from the results shown here.

The oscillating gradient dMRI technique also has certain limitations. Limitations of current gradient hardware place a constraint on the minimum achievable diffusion time for a given b -value (Table 1). Decreasing the diffusion time further entails increasing the gradient oscillation frequency, which, in turn, requires even stronger gradient amplitudes to maintain a constant b -value. The demand for strong gradient amplitudes also makes this technique technically difficult to implement on current clinical scanners. The long diffusion gradient durations necessary for oscillating gradient waveforms result in lengthening the echo time for image acquisitions, thereby worsening the signal-to-noise ratio (SNR) in the already low SNR technique. Additionally, the need to acquire diffusion data at multiple frequencies with this technique leads to long total acquisition times. With further understanding of the contrast properties and their frequency-dependent characteristics, it may be possible to acquire data at fewer frequencies, thereby reducing the total imaging time and improving feasibility for application to *in vivo* studies. As for oscillating gradient DTI, it inherits the limitations of DTI as a simplified model for complex tissue structures, such as crossing or branching fibers. In many cases, it is not necessary to acquire the complete tensor dataset if ADC measurement is sufficient, which can reduce the total time required. The ADC

measurements obtained at different frequencies in our study were fitted to a linear model, since within the relatively narrow frequency domain used in our study the frequency-dependence of ADC can be approximated by a linear curve. The diffusion spectrum over a broader frequency range, however, is nonlinear (21,49), and the linear model will have to be replaced by appropriate nonlinear models over wider frequency ranges.

To summarize, this study demonstrated unique tissue contrasts in the mouse brain using oscillating gradient dMRI. The distinctive delineation of densely packed neuronal regions with this technique has interesting implications for studies investigating neuronal defects in the hippocampus or cerebellum in mouse models of related disorders. The finding that frequency-dependent DTI contrasts are sensitive to microstructural white matter changes in the cuprizone model also renders this technique useful for applications in mouse models of white matter disorders.

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References

1. Chenevert TL, McKeever PE, Ross BD. Monitoring early response of experimental brain tumors to therapy using diffusion magnetic resonance imaging. *Clin Cancer Res.* 1997; 3(9):1457–1466. [PubMed: 9815831]
2. Moffat BA, Chenevert TL, Lawrence TS, Meyer CR, Johnson TD, Dong Q, Tsien C, Mukherji S, Quint DJ, Gebarski SS, Robertson PL, Junck LR, Rehemtulla A, Ross BD. Functional diffusion map: a noninvasive MRI biomarker for early stratification of clinical brain tumor response. *Proc Natl Acad Sci U S A.* 2005; 102(15):5524–5529. [PubMed: 15805192]
3. Moseley ME, Cohen Y, Mintorovitch J, Chileuitt L, Shimizu H, Kucharczyk J, Wendland MF, Weinstein PR. Early detection of regional cerebral ischemia in cats: comparison of diffusion- and T2-weighted MRI and spectroscopy. *Magn Reson Med.* 1990; 14(2):330–346. [PubMed: 2345513]
4. Cercignani M, Bozzali M, Iannucci G, Comi G, Filippi M. Magnetisation transfer ratio and mean diffusivity of normal appearing white and grey matter from patients with multiple sclerosis. *J Neurol Neurosurg Psychiatry.* 2001; 70(3):311–317. [PubMed: 11181851]
5. Pierpaoli C, Barnett A, Pajevic S, Chen R, Penix LR, Virta A, Basser P. Water diffusion changes in Wallerian degeneration and their dependence on white matter architecture. *Neuroimage.* 2001; 13(6 Pt 1):1174–1185. [PubMed: 11352623]
6. Le Bihan D. Looking into the functional architecture of the brain with diffusion MRI. *Nat Rev Neurosci.* 2003; 4(6):469–480. [PubMed: 12778119]
7. Basser P, Pierpaoli C. Microstructural and physiological features of tissues elucidated by quantitative-diffusion-tensor MRI. *J Magn Reson B.* 1996; 111:209–219. [PubMed: 8661285]
8. Mori S, Zhang J. Principles of diffusion tensor imaging and its applications to basic neuroscience research. *Neuron.* 2006; 51(5):527–539. [PubMed: 16950152]
9. Callagan, PT. Principles of Nuclear Magnetic Resonance Microscopy. Oxford University Press; New York: 1991.
10. Beaulieu C. The basis of anisotropic water diffusion in the nervous system - a technical review. *NMR Biomed.* 2002; 15(7-8):435–455. [PubMed: 12489094]
11. Kim S, Chi-Fishman G, Barnett AS, Pierpaoli C. Dependence on diffusion time of apparent diffusion tensor of ex vivo calf tongue and heart. *Magn Reson Med.* 2005; 54(6):1387–1396. [PubMed: 16265644]
12. Latour LL, Svoboda K, Mitra PP, Sotak CH. Time-dependent diffusion of water in a biological model system. *Proc Natl Acad Sci U S A.* 1994; 91(4):1229–1233. [PubMed: 8108392]

13. Niendorf T, Norris DG, Leibfritz D. Detection of apparent restricted diffusion in healthy rat brain at short diffusion times. *Magn Reson Med*. 1994; 32(5):672–677. [PubMed: 7808271]
14. Ozarslan E, Basser PJ, Shepherd TM, Thelwall PE, Vemuri BC, Blackband SJ. Observation of anomalous diffusion in excised tissue by characterizing the diffusion-time dependence of the MR signal. *J Magn Reson*. 2006; 183(2):315–323. [PubMed: 16962801]
15. Stejskal EO, Tanner JE. Spin Diffusion Measurements: Spin Echoes in the Presence of a Time-Dependent Field Gradient. *J Chem Phys*. 1965; 42(1):288.
16. Callaghan PT, Stepisnik J. Generalized analysis of motion using magnetic field gradients *Advances in Magnetic and Optical Resonance*. 1996; 19:325–388.
17. Parsons EC, Does MD, Gore JC. Modified oscillating gradient pulses for direct sampling of the diffusion spectrum suitable for imaging sequences. *Magn Reson Imaging*. 2003; 21(3-4):279–285. [PubMed: 12850719]
18. Schachter M, Does MD, Anderson AW, Gore JC. Measurements of restricted diffusion using an oscillating gradient spin-echo sequence. *J Magn Reson*. 2000; 147(2):232–237. [PubMed: 11097814]
19. Stepisnik J. Analysis of NMR self-diffusion measurements by a density-matrix calculation. *Physica B & C*. 1981; 104(3):350–364.
20. Stepisnik J. Time-dependent self-diffusion by NMR spin-echo. *Physica B*. 1993; 183:343–350.
21. Parsons EC Jr. Does MD, Gore JC. Temporal diffusion spectroscopy: theory and implementation in restricted systems using oscillating gradients. *Magn Reson Med*. 2006; 55(1):75–84. [PubMed: 16342147]
22. Gore JC, Xu J, Colvin DC, Yankeelov TE, Parsons EC, Does MD. Characterization of tissue structure at varying length scales using temporal diffusion spectroscopy. *NMR Biomed*. 2010; 23(7):745–756. [PubMed: 20677208]
23. Colvin DC, Yankeelov TE, Does MD, Yue Z, Quarles C, Gore JC. New insights into tumor microstructure using temporal diffusion spectroscopy. *Cancer Res*. 2008; 68(14):5941–5947. [PubMed: 18632649]
24. Does MD, Parsons EC, Gore JC. Oscillating gradient measurements of water diffusion in normal and globally ischemic rat brain. *Magn Reson Med*. 2003; 49(2):206–215. [PubMed: 12541239]
25. Matsushima GK, Morell P. The neurotoxicant, cuprizone, as a model to study demyelination and remyelination in the central nervous system. *Brain Pathol*. 2001; 11(1):107–116. [PubMed: 11145196]
26. Smith BR, Huff DS, Johnson GA. Magnetic resonance imaging of embryos: an Internet resource for the study of embryonic development. *Comput Med Imaging Graph*. 1999; 23(1):33–40. [PubMed: 10091866]
27. Benveniste H, Blackband S. MR microscopy and high resolution small animal MRI: applications in neuroscience research. *Prog Neurobiol*. 2002; 67(5):393–420. [PubMed: 12234501]
28. Xu J, Does MD, Gore JC. Sensitivity of MR diffusion measurements to variations in intracellular structure: effects of nuclear size. *Magn Reson Med*. 2009; 61(4):828–833. [PubMed: 19205020]
29. Aggarwal M, Mori S, Shimogori T, Blackshaw S, Zhang J. Three-dimensional diffusion tensor microimaging for anatomical characterization of the mouse brain. *Magn Reson Med*. 2010; 64(1):249–261. [PubMed: 20577980]
30. Jiang H, van Zijl PC, Kim J, Pearlson GD, Mori S. DtiStudio: resource program for diffusion tensor computation and fiber bundle tracking. *Comput Methods Programs Biomed*. 2006; 81(2):106–116. [PubMed: 16413083]
31. Baschong W, Suetterlin R, Laeng RH. Control of autofluorescence of archival formaldehyde-fixed, paraffin-embedded tissue in confocal laser scanning microscopy (CLSM). *J Histochem Cytochem*. 2001; 49(12):1565–1572. [PubMed: 11724904]
32. Paxinos, G.; Franklin, K. *The Mouse Brain in Stereotaxic Coordinates*: Compact. Second Edition. Academic Press; 2003.
33. Stepisnik J, Callaghan PT. Low-frequency velocity correlation spectrum of fluid in a porous media by modulated gradient spin echo. *Magn Reson Imaging*. 2001; 19(3-4):469–472. [PubMed: 11445332]

34. Stepisnik J, Lasic S, Mohoric A, Sersa I, Sepe A. Spectral characterization of diffusion in porous media by the modulated gradient spin echo with CPMG sequence. *J Magn Reson.* 2006; 182(2): 195–199. [PubMed: 16844392]
35. Paxinos, G. *The Rat Nervous System.* Academic Press; New York: 2004.
36. Zhao C, Teng EM, Summers RG Jr, Ming GL, Gage FH. Distinct morphological stages of dentate granule neuron maturation in the adult mouse hippocampus. *J Neurosci.* 2006; 26(1):3–11. [PubMed: 16399667]
37. Diaz-Cintra S, Yong A, Aguilar A, Bi X, Lynch G, Ribak CE. Ultrastructural analysis of hippocampal pyramidal neurons from apolipoprotein E-deficient mice treated with a cathepsin inhibitor. *J Neurocytol.* 2004; 33(1):37–48. [PubMed: 15173631]
38. Kim SU. Observations on cerebellar granule cells in tissue culture. A silver and electron microscopic study. *Z Zellforsch Mikrosk Anat.* 1970; 107(4):454–465. [PubMed: 5448478]
39. Wimer RE, Wimer CC. A biometrical-genetic analysis of granule cell number in the area dentata of house mice. *Brain Res.* 1981; 254(1):129–140. [PubMed: 7272765]
40. Wimer RE, Wimer CC, Chernow CR, Balvanz BA. The genetic organization of neuron number in the pyramidal cell layer of hippocampal regio superior in house mice. *Brain Res.* 1980; 196(1):59–77. [PubMed: 7397531]
41. Stidworthy MF, Genoud S, Suter U, Mantei N, Franklin RJ. Quantifying the early stages of remyelination following cuprizone-induced demyelination. *Brain Pathol.* 2003; 13(3):329–339. [PubMed: 12946022]
42. Song SK, Yoshino J, Le TQ, Lin SJ, Sun SW, Cross AH, Armstrong RC. Demyelination increases radial diffusivity in corpus callosum of mouse brain. *Neuroimage.* 2005; 26(1):132–140. [PubMed: 15862213]
43. Sun SW, Liang HF, Trinkaus K, Cross AH, Armstrong RC, Song SK. Noninvasive detection of cuprizone induced axonal damage and demyelination in the mouse corpus callosum. *Magn Reson Med.* 2006; 55(2):302–308. [PubMed: 16408263]
44. Xie M, Tobin JE, Budde MD, Chen CI, Trinkaus K, Cross AH, McDaniel DP, Song SK, Armstrong RC. Rostrocaudal analysis of corpus callosum demyelination and axon damage across disease stages refines diffusion tensor imaging correlations with pathological features. *J Neuropathol Exp Neurol.* 69(7):704–716. [PubMed: 20535036]
45. Pfeuffer J, Flogel U, Dreher W, Leibfritz D. Restricted diffusion and exchange of intracellular water: theoretical modelling and diffusion time dependence of ¹H NMR measurements on perfused glial cells. *NMR Biomed.* 1998; 11(1):19–31. [PubMed: 9608585]
46. Clark CA, Le Bihan D. Water diffusion compartmentation and anisotropy at high b values in the human brain. *Magn Reson Med.* 2000; 44(6):852–859. [PubMed: 11108621]
47. Thelwall PE, Shepherd TM, Stanisz GJ, Blackband SJ. Effects of temperature and aldehyde fixation on tissue water diffusion properties, studied in an erythrocyte ghost tissue model. *Magn Reson Med.* 2006; 56(2):282–289. [PubMed: 16841346]
48. Shepherd TM, Thelwall PE, Stanisz GJ, Blackband SJ. Aldehyde fixative solutions alter the water relaxation and diffusion properties of nervous tissue. *Magn Reson Med.* 2009; 62(1):26–34. [PubMed: 19353660]
49. Xu J, Does MD, Gore JC. Quantitative characterization of tissue microstructure with temporal diffusion spectroscopy. *J Magn Reson.* 2009; 200(2):189–197. [PubMed: 19616979]

FIG. 1.

The gradient waveforms $G(t)$ used in the PGSE sequence (solid curve in **A**) and OGSE sequences with oscillation frequencies of 50 Hz, 100 Hz, and 150 Hz (solid curves in **B-D**, respectively), and their corresponding $F(f)$ (**E**). The magnitudes of the gradient waveforms have been normalized to the range of -1 to 1 , and the durations are equal to the echo time (50 ms). The dotted curves in figures **A-D** have polarity opposite to that of the solid curves in the second half, which represents the effect of the refocusing pulse. Figure **E** shows the normalized results of Fourier transform of the gradient waveforms ($F(f)$) for the PGSE (black) and OGSE (blue: 50Hz, green: 100 Hz, red: 150 Hz) sequences (dotted curves in **A-D**).

FIG. 2.

Coronal T_2 -weighted and diffusion tensor images of an *ex vivo* mouse brain acquired using both PGSE and OGSE sequences at 50 Hz, 100 Hz, and 150 Hz. Anatomical structures are labeled in the T_2 -weighted image. Maps of ADC, FA and direction-encoded colormap (DEC) images are generated from the diffusion tensor. The unit of the ADC is mm^2/s . The three color arrows at the lower right illustrate the color scheme in the DEC images which uses red for the medial-lateral axis, green for the anterior-posterior axis, and blue for the superior-inferior axis. Structural abbreviations are: cc: corpus callosum; cp: cerebral peduncle; CX: cerebral cortex; H: hippocampus.

FIG. 3.

Values of ADC, FA, parallel diffusivity ($\lambda_{||}$), and perpendicular diffusivity ($\lambda_{\perp}$) measured in major structures using the PGSE and OGSE sequences. Data are plotted as mean $\pm$ standard deviation for four mouse brains. Structural abbreviations are: cp: cerebral peduncle; fi: fimbria; gcc: genu of the corpus callosum; mcx: motor cortex; scc: splenium of the corpus callosum; scx: sensory cortex.

FIG. 4.

Enhanced tissue contrasts in the mouse hippocampus and cerebellum in oscillating gradient diffusion tensor images. **A:** Coronal and sagittal T_2 -weighted images and enlarged ADC images of the hippocampus acquired using the PGSE and OGSE sequences and corresponding Nissl stained sections. **B:** mid-sagittal T_2 -weighted images and ADC images of the cerebellum acquired using the PGSE and OGSE sequences and corresponding Nissl stained section. The Nissl stained sections are from the Paxinos' mouse brain atlas. **C:** changes in mean ADC values of selected structures acquired using the PGSE and OGSE sequences. Structural abbreviations are: CBGr: cerebellar granule cell layer; CBML: cerebellar molecular layer; GrDG: granule cell layer of the dentate gyrus; Py: pyramidal cell layer of the hippocampus, CA1: CA1 subfield of the hippocampus.

FIG. 5.

The spatial organization of neurons and axons in the mouse hippocampus (A & B) and cerebellum (C & D) in immunostained sections. The sections were stained with DAPI (blue, for nuclei) and SMI-31 (green, for phosphorylated neurofilament in axons). B and D are higher magnification (20X) images of the regions outlined by the dashed boxes in A and C (10X), respectively. Scale bars = 100 μ m. Structural abbreviations are: CBGr: cerebellar granule cell layer; CBML: cerebellar molecular layer; GrDG: granule cell layer of the dentate gyrus; Py: pyramidal cell layer of the hippocampus.

FIG. 6. Comparison of T2-weighted images, maps representing the linear fits of ADC versus frequency (Δ_f ADC), and Nissl stained sections of the mouse brain. Three coronal sections (A), one sagittal section (B), and one horizontal section (C) are shown. The Nissl stained sections are from the Paxinos' mouse brain atlas. Arrows in the figure point to regions that show enhancement in the cerebral cortex (1), piriform cortex (2), dentate gyrus (3), olfactory bulb (4), and cerebellum (5).

FIG. 7.

A: T2-weighted images, maps of perpendicular diffusivity ($\lambda_{\perp}$) measured using PGSE sequence, and maps of the rate of change of perpendicular diffusivity with frequency ($\Delta_f \lambda_{\perp}$) measured using the OGSE sequence, of the corpus callosum in the control mice (0 week), mice after 4 weeks of cuprizone diet, and mice after 6 of weeks cuprizone diet. **B:** Plots of FA, $\lambda_{\perp}$, and $\Delta_f \lambda_{\perp}$ of the caudal corpus callosum. *: $p < 0.005$.

FIG. 8.

Organization of axons in the mouse corpus callosum and infiltration of activated microglia at 4 weeks after cuprizone treatment. Coronal immunostained sections of the caudal corpus callosum of a control mouse and mice after 4 and 6 weeks of cuprizone treatment are shown. The sections were stained with iba-1 (red, for activated microglia) and SMI-31 (green, for phosphorylated neurofilament in axons). Disruption of axons at the 4 and 6 week time points can be seen. Swelling of the corpus callosum, indicated by an increase in the thickness, is apparent at the 4 week time point. Structural abbreviations are: cc: corpus callosum.

Table 1

Diffusion-weighting parameters for the PGSE and OGSE experiments

	f (Hz)	t_p (ms)	δ in PGSE /N in OGSE	b (s/mm²)	G (mT/m)
PGSE	0	15	5 ms	702.2	217
	50	5	20 ms/N=1	702.5	235
OGSE	100	2.5	20 ms/N=2	701.2	454
	150	1.67	20 ms/N=3	701.9	674

Table 2

Diffusivity, ADC and FA measurements in the cerebellar molecular (CBML) and granule cell (CBGr) layers at different oscillation frequencies. Data are shown as mean $\pm$ standard deviation for four mouse brains

f (Hz)	$\lambda_d (10^{-4} \text{ mm}^2/\text{s})$		$\lambda_L (10^{-4} \text{ mm}^2/\text{s})$		ADC ($10^{-4} \text{ mm}^2/\text{s})$		FA	
	CBML	CBGr	CBML	CBGr	CBML	CBGr	CBML	CBGr
0	6.67 $\pm$ 0.61	5.76 $\pm$ 1.23	4.12 $\pm$ 0.56	4.01 $\pm$ 1.17	4.97 $\pm$ 0.41	4.65 $\pm$ 0.98	0.31 $\pm$ 0.08	0.27 $\pm$ 0.04
50	6.72 $\pm$ 0.74	7.28 $\pm$ 0.77	4.15 $\pm$ 0.41	5.23 $\pm$ 0.78	5.01 $\pm$ 0.42	5.96 $\pm$ 0.64	0.31 $\pm$ 0.05	0.23 $\pm$ 0.02
100	7.10 $\pm$ 0.98	9.46 $\pm$ 0.62	4.41 $\pm$ 0.41	7.07 $\pm$ 0.49	5.31 $\pm$ 0.38	7.95 $\pm$ 0.39	0.30 $\pm$ 0.09	0.20 $\pm$ 0.04
150	7.33 $\pm$ 0.70	11.2 $\pm$ 0.35	4.69 $\pm$ 0.40	8.55 $\pm$ 0.38	5.57 $\pm$ 0.27	9.55 $\pm$ 0.47	0.28 $\pm$ 0.08	0.17 $\pm$ 0.01

Table 3

Fitted ADC versus frequency slopes (Δ_f ADC) for different grey matter regions in the mouse brain. Data are shown as mean $\pm$ standard deviation (n=4). Structural abbreviations are: CBGr: cerebellar granule cell layer, CBml: cerebellar molecular layer, GrDG: granule cell layer of the dentate gyrus, Py: hippocampal pyramidal cell layer

Brain regions	Δ_f ADC (10^{-6} mm ²)
Motor cortex	0.69 $\pm$ 0.13
Sensory cortex	0.71 $\pm$ 0.11
Piriform cortex	1.54 $\pm$ 0.07
CBGr	3.69 $\pm$ 0.15
CBml	0.71 $\pm$ 0.04
GrDG	2.85 $\pm$ 0.07
Py	1.74 $\pm$ 0.08