Published in final edited form as:

NMR Biomed. 2017 January ; 30(1): . doi:10.1002/nbm.3671.

Probing metabolite diffusion at ultra-short time scales in the mouse brain using optimized oscillating gradients and “short” echo time diffusion-weighted MR spectroscopy

Clémence Ligneul^{1,2} and Julien Valette^{1,2,*}

¹Commissariat à l'Energie Atomique et aux Energies Alternatives (CEA), Direction de la Recherche Fondamentale (DRF), Institut d'Imagerie Biomédicale (I2BM), MIRCen, F-92260 Fontenay-aux-Roses, France

²Centre National de la Recherche Scientifique (CNRS), Université Paris-Sud, Université Paris-Saclay, UMR 9199, Neurodegenerative Diseases Laboratory, F-92260 Fontenay-aux-Roses, France

Abstract

Measuring diffusion at ultra-short time scales may yield information about short-range intracellular structure and cytosol viscosity. However, reaching such time scales usually requires oscillating gradients, which in turn imply long echo times TE. Here we propose a new kind of stretched oscillating gradients that allow increasing diffusion-weighting b while preserving spectral and temporal properties of the gradient modulation. We used these optimized gradients to measure metabolite diffusion in the mouse brain down to effective diffusion times of 1 ms while keeping TE relatively short (60 ms). At such TE, a significant macromolecule signal could still be observed and used as an internal reference of approximately null diffusivity, which proved critical to discard datasets corrupted by some motion artifact. The methods introduced here may be useful to improve the accuracy and precision of metabolite ADC measurements with oscillating gradients.

Keywords

magnetic resonance spectroscopy; metabolite; diffusion; brain; oscillating gradient; macromolecule; motion artifact

Introduction

Diffusion-weighted magnetic resonance spectroscopy (DW-MRS) has the enormous potential to non-invasively probe intracellular space organization, due to the cell-specific compartmentation of most metabolites (for review see (1–3)). The apparent diffusion coefficient (ADC) that can be quantified for metabolites is related to their quadratic displacement during the diffusion time t_d . Thus, DW-MRS is potentially sensitive to any

*Corresponding author: Julien Valette, Commissariat à l'Energie Atomique et aux Energies Alternatives (CEA), Bâtiment 61, 8 Route du Panorama, 92260 Fontenay-aux-Roses, France, julien.valette@cea.fr.

phenomenon affecting the quadratic displacement during t_d including viscosity, subcellular compartmentation, presence of organelles, and cell shape and size. Up to now, most works have been performed in a relatively narrow range of diffusion times (from 11.7 ms (4) to 242 ms (5)). In recent years, we have engaged in the exploration of metabolite diffusion over unprecedented times scales, from very short times (~ 1 ms) in the rat brain (6), to very long times (~ 1 -2 second) in the mouse (7), primate (7,8) and human brain (9), in order to probe the intracellular environment at various spatial scales. From these studies, the following pattern has emerged: as t_d is increased, metabolite ADC quickly decreases at short times, while it then tends to stabilize at a plateau at longer times (although with a very slight tendency to keep decreasing). Taken together, these data suggest that metabolites are encountering numerous new obstacles at short time scales (e.g. organelles or membranes of cellular processes), while they are then relatively free to diffuse along the direction of cellular process (i.e. they barely encounter new kinds of hindering structures as they diffuse along fibers). In our opinion, this illustrates the potential of studying metabolites diffusion at unconventional diffusion times.

One of the limitation of our work in the rat brain (6) at very short t_d or more precisely at very high frequencies as oscillating gradients were used, was the relatively low signal-to-noise ratio (SNR) in the spectra due to the long echo times (TE=154 ms), largely imposed by the necessity to use long oscillating gradients with several successive dephasing-rephasing periods to reach sufficient diffusion-weighting. Low SNR resulted in large standard deviation on ADC, and the use of long TE also reduced the set of observable metabolites to the most prominent singlet peaks (N-acetyl-aspartate NAA, total creatine tCr and choline compounds tCho). The goal of the present work is to revisit metabolite diffusion at very short time scales, by proposing a short TE strategy allowing the observation of a larger number of metabolites, with a better precision. First, from the apodized-cosine originally proposed in (10), we derive a new class of oscillating gradient, by stretching cosine's lobes to maximize diffusion-weighting for a given period and gradient duration, while well preserving the gradient modulation spectrum. Using these gradients, we then perform DW-MRS experiments at TE=60 ms in the mouse brain at 11.7 T. One unexpected result is that some intermittent motion artifact seems to occur at some particular frequencies, as evidenced when quantifying macromolecule (MM) signal, which can be considered as an internal reference of almost null diffusivity. We propose to use MM signal as a quality control to retrospectively discard spectra corrupted by this motion artifact. In the end, we are able to measure ADC for NAA, tCr, tCho, myo-inositol (Ins) and taurine (Tau) for frequencies ranging from 35 Hz to 252 Hz (corresponding to effective t_d ranging from 0.99 ms to 7.1 ms) in the mouse brain.

Theory

Oscillating gradients

Oscillating gradients have been used to probe water diffusion at short time scales *in vitro* or *in vivo*. The maximal gradient strength constitutes an important hardware limitation to keep a good diffusion contrast at very short diffusion times / high frequencies. Since water diffuses quite fast ($D_{\text{free}} \sim 2 \mu\text{m}^2/\text{ms}$ at 20°C), very high frequencies have already been

reached with sufficient diffusion contrast *in vitro* or *in vivo* even at low b-values, around $b_{\max}=0.4 \text{ ms}/\mu\text{m}^2$ ((10–12)). In contrast, metabolites are diffusing much slower than water, so that achieving good diffusion contrast requires higher b values. Diffusion-weighted spectroscopy at very high frequencies is therefore a methodological challenge in terms of signal and diffusion contrast, and appeals for solutions to increase the maximal reachable b value within the same maximal gradient strength.

The most optimized existing oscillating gradient shape in terms of frequency selection is the apodized cosine gradient, defined in (10) as a sine-edged cosine-modulated gradient, that we used in a previous DW-MRS work (6). To increase $b_{\max}$ we propose to stretch the lobes of this gradient shape. This can be achieved by introducing a stretching exponent α resulting in an increased area under each lobe, as detailed in the above gradient waveform equation:

$$G(t) = (-1)^{k+1} G_{\max} \cos \left(\frac{\pi}{2} \left(\frac{8Nt}{T_g} - k \right) - 1 \right)^\alpha \quad \text{for } 0 \leq t < \frac{T_g}{4N}$$

$$G(t) = (-1)^{k+1} G_{\max} \cos \left(\frac{\pi}{2} \left(2 \left(\frac{4Nt}{T_g} - k \right) - 1 \right)^\alpha \right) \quad \text{for } \frac{T_g}{4N} \leq t < T_g - \frac{T_g}{4N}, k = E \left(\frac{4Nt}{T_g} \right)$$

$$G_{\max} \cos \left(\frac{\pi}{2} \left(\frac{8Nt}{T_g} - 8N + 1 \right)^\alpha \right) \quad \text{for } T_g - \frac{T_g}{4N} \leq t < T_g \quad (1)$$

N is the number of periods, T_g is the total duration of the gradient waveform, $G_{\max}$ is the amplitude of the gradient and α is the stretching exponent. $\alpha=1$ corresponds to the usual apodized cosine gradient introduced in (10). Note that stretched cosine gradient tends to an oscillating crenel when α tends to infinity (which would be equivalent to the “trapezoid-cosine” waveform (13) with infinitely short rise time).

With oscillating gradients, the best approach to describe diffusion-weighting is to work in the frequency domain, as originally proposed by Stepisnik and Callaghan (14,15), by considering two important objects: the diffusion spectrum $D(\omega)$ (which is the Fourier transform of particles' velocity autocorrelation function along gradient's direction), and the gradient modulation spectrum $F(\omega)$ (the Fourier transform of the time integral of gradient waveform $G(t)$). This allows evaluating signal attenuation S/S_0 (10):

$$\ln \left(\frac{S}{S_0} \right) = - \frac{1}{\pi} \int_0^\infty |F(\omega)|^2 D(\omega) d\omega \quad (2)$$

Long oscillating gradients yield $F(\omega)$ characterized by narrow peaks, allowing clean probing of $D(\omega)$ at different frequencies. Gradient waveform and spectrum $|F(\omega)|^2$ for $\alpha=2, 4$ and ∞ (numerically calculated) are represented in Figure 1A, and 1B, and compared to $\alpha=1$, for a gradient duration of 20 ms and $N=3$. Note that, on Figure 1B, the $|F(\omega)|^2$ functions were normalized (i.e. divided by their integral) to facilitate comparison. The calculation of the gradient modulation spectrum was actually done for two 20-ms gradient waveforms surrounding a 2-ms refocusing RF pulse, as in a realistic sequence (see Methods). The gradient modulation spectra are extremely similar, in terms of the position of the main and secondary peaks, for the usual and stretched cosine gradients. Hence we can safely assume that the frequency selections are identical in good approximation. The reader can refer to the appendix to be convinced that this approximation is still relevant even in the worst case

scenario (oscillating crenel, i.e. $\alpha=\infty$). Because α does not strongly affect the selection properties of the waveform, we will use the same relationship as given in (11) between the number of periods (or the frequency) and the effective diffusion time for an apodized cosine:

$t_d = \frac{1}{4\omega}$. Now, whatever α , some secondary lobes appear on the gradient modulation spectra, due to the relatively low N examined here (and used below) and the presence of a 2-ms gap between the two waveforms, which degrade the perfect frequency selection ideally achieved at $\omega=N/T_g$. This can be visualized on Figure 1C, which is a zoom around the main peak, showing the secondary lobes (for different α). Although this is far from capturing all the information conveyed in the gradient modulation spectrum, we propose to compute an effective sampled frequency using a weighted-average frequency, to account for the influence of all lobes in the actual gradient modulation spectrum:

$$\omega_{\text{eff}} = \frac{\int_0^\infty \omega |F(\omega)|^2 d\omega}{\int_0^\infty |F(\omega)|^2 d\omega} \quad (3)$$

Gain in b

The integral of $|F|^2$ (or equivalently of k^2 according to Parseval's theorem), i.e. b , is increasing with α . We numerically quantified the gain in b as a function of α , $s(\alpha)$, represented in Figure 1D. The increase is important for low α values (gain of 1.56 for $\alpha=4$ compared to $\alpha=1$), but then tends to become marginal for higher α (gain from 1.56 to 1.72 for $\alpha=4$ to $\alpha=\infty$).

Methods

Experimental setup

Stretched oscillating gradients were implemented within a diffusion-weighted asymmetric LASER (Localization by Adiabatic SElective Refocusing) sequence as described in (6), on a horizontal 11.7 T Bruker scanner running with Paravision 6.0. The maximal gradient strength is $G_{\text{max}}=752$ mT/m on each axis, with 100- μ s rise time. A quadrature surface cryogenic antenna was used for experiments in the mouse brain. The hyperbolic secant pulses used for refocusing in LASER had a 2-ms duration (corresponding to 10-kHz bandwidth).

To maximize metabolite and macromolecule signal, TE was chosen to be relatively short (TE=60 ms) with a gradient duration T_g of 20 ms for each waveform (i.e. a total diffusion gradient duration of 40 ms, considering the two waveform positioned symmetrically around the first refocusing pulse). Repetition time was set to 3000 ms. Water suppression was achieved with a VAPOR module. Diffusion-weighting was applied on all three axes simultaneously, using a stretching factor $\alpha=4$. We considered it as a reasonable compromise between getting a significant increase in diffusion-weighting while remaining below maximal gradient ramp. In a first set of experiments, metabolite signal was acquired at "low frequencies" ($\omega_{\text{eff}} \approx 35$ Hz and $\omega_{\text{eff}} \approx 99$ Hz, corresponding to $N=1$ and 2 periods) and "high" b (up to $b=8$ ms/ μm^2 with 1 ms/ μm^2 -step). In a second set of experiments, signal was

acquired at low b (0 and 1.2 ms/ μm^2) but going to higher frequencies (ω_{eff} =35, 99, 150, 201 and 252 Hz, corresponding to N from 1 to 5). The maximum gradient slew rate actually reached using the stretched gradients (at $N=5$ during the first and last half lobes, which are the most constraining) was actually close to the maximal slew rate achievable. Note that it corresponds to a magnetic field variation of 120 T/s (considering all three axes) achieved during ~ 1 ms at 5 cm from the isocenter, i.e. at the position of hind legs. For comparison, the field variation can be estimated to be 130 T/s (considering all three axes) during ~ 1 ms at 1.5 meter of the isocenter in Van et al. (13), which was low enough to avoid peripheral nerve stimulation when using oscillating gradients in Humans. Hence it is unlikely that peripheral nerve stimulation occurred in our experiments. Each acquisition consisted in 64 scans stored individually. Voxel dimension was $6 \times 2.4 \times 5 \text{ mm}^3$ (see insert in Figure 2).

Phantom validation

To assess the validity of this new gradient shape, in particular regarding the b -value, experiments were performed on a water phantom at 20°C using $\alpha=4$ for the five frequencies and experimental conditions we used in vivo. Spectra have a normal aspect, without detectable eddy currents. Signal attenuation has a perfect mono-exponential behavior up to $b=1.2 \text{ ms}/\mu\text{m}^2$, yielding $D_{\text{water}}=2.16 \pm 0.02 \mu\text{m}^2/\text{ms}$, in good agreement with literature. Note that we found exactly the same values using a standard apodized cosine.

Animals

Experiments were performed on five male C57BL/6 mice (body weight ~ 28 -30 g). Protocol was approved by the local ethical committee of the Commissariat à l'Energie Atomique (French Atomic Energy Commission). All animal experimental procedures were performed in accordance with the recommendations of the European Union (2010/63/EU) for care and use of laboratory animals, and conformed to the ethical guidelines of the French Ministry of Agriculture and Forests. Mice were anesthetized with 1.2-1.5% isoflurane in a 1:1 mixture of air and dioxygen (1 L/min). Animals were placed on a stereotaxic bed with a bit and two ear bars to prevent head motion. Mice temperature was monitored with an endorectal probe and maintained at 37°C with regulated water flow, and respiratory rate was continuously monitored using PC SAM software (Small Animal Instruments, Inc., Stony Brook, NY, USA) during scanning. Thirty experiments were performed. For most experiments all frequencies could be acquired, however for a few experiments the acquisition had to be stopped before the end, so that spectra for some frequencies were actually acquired less than thirty times. The number of measurements for each frequency is given in Table 1. At the end of the experiment, animals were placed on a heating pad and kept under observation until they were fully awake.

Data analysis

Scan-to-scan phase correction was performed before summing individual scans of each acquisition on Matlab. Spectra were analyzed with LCModel (16), using a basis set generated with home-made routines based on density matrix formalism. A macromolecule (MM) spectrum was acquired using the metabolite nulling technique combined with diffusion-weighting ($\text{TI}=780 \text{ ms}$, $b=10 \text{ ms}/\mu\text{m}^2$ at $\omega_{\text{eff}}=35 \text{ Hz}$) to yield cleaner metabolite cancellation. Residual metabolite signals coming from resonances with shorter T_1 relaxation

were reduced through Hankel Lanczos singular value decomposition (HLSVD) as in (17). The resulting spectrum was included in the LCModel basis set. Signal could be reliably quantified (Cramér-Rao lower bounds CRLB<5%) for NAA, tCr, tCho, Ins and Tau. Glutamate was not properly quantified at this specific TE. ADC was evaluated by log-linear regression for the “high b” experiments where several b-values were available, or otherwise

with the simple formula $ADC = -\frac{1}{b} \ln \left(\frac{s}{s_0} \right)$.

Results

Data quality

All spectra were acquired with a cryogenic antenna at 11.7 T, yielding good signal-to-noise ratio (SNR~170 on NAA, without diffusion-weighting) and good quantification precision. Indeed, total NAA and total creatine were quantified on LCModel with CRLB of 0% or 1%. tCho, Tau and Ins were evaluated at about 1 or 2% CRLB. No eddy current could be detected. We actually checked that eddy current correction as we usually perform in post processing had no impact on spectral quality, quantification and ADC at all frequencies, and was therefore not necessary.

Signal attenuation at high diffusion-weighting

Figure 2A present stack plots for spectra at different diffusion-weighting, at 35 Hz and 99 Hz. Signal attenuation as quantified for NAA, tCr, tCho, Ins and Tau is shown in Figure 2B. Metabolite signal attenuation is clearly dependent on the chosen frequency, especially at higher b-values. Signal attenuation appears relatively close to mono-exponentially in this range of b-values ($R^2 > 0.98$ at $\omega_{eff}=35$ Hz and $R^2 > 0.99$ at $\omega_{eff}=99$ Hz for all metabolites, except for Tau which seems noisier, especially at the highest b). Log-linear fit of metabolite signal gives the following ADC at $\omega_{eff}=35$ Hz and $\omega_{eff}=99$ Hz, respectively: 0.083 and 0.108 $\mu\text{m}^2/\text{ms}$ for NAA; 0.103 and 0.125 $\mu\text{m}^2/\text{ms}$ for tCr; 0.100 and 0.133 $\mu\text{m}^2/\text{m}$ for tCho; 0.106 and 0.117 $\mu\text{m}^2/\text{ms}$ for Tau; 0.106 and 0.134 $\mu\text{m}^2/\text{ms}$ for Ins.

The macromolecule signal attenuation appears to be scattered. To get an estimation of the ADC, a linear fit was done between $b=0$ and $b=5$ $\text{ms}/\mu\text{m}^2$, yielding $ADC=0.028$ $\mu\text{m}^2/\text{ms}$ at $\omega_{eff}=35$ Hz, and $ADC=0.024$ $\mu\text{m}^2/\text{ms}$ at $\omega_{eff}=99$ Hz. ADC at both frequencies are quite close to MM ADC as measured in the mouse brain in a recent study (18) (0.016 ± 0.005 $\mu\text{m}^2/\text{ms}$ at $t_d=63$ ms, as determined by a linear fit between $b=0$ and $b=5$ $\text{ms}/\mu\text{m}^2$). The value is slightly higher than (17) measured in the rat brain ($ADC=0.006$ $\mu\text{m}^2/\text{ms}$), but in that paper the authors did a linear fit up to high b values (50 $\text{ms}/\mu\text{m}^2$), which might lead to ADC under-evaluation.

Signal attenuation at high-frequency, and possibility to use macromolecules as an internal reference

For experiments at high-frequency (>100 Hz), we noticed that metabolite signal attenuation was sometimes much stronger than usual (Figure 3), resulting in very high ADC. This phenomenon pointed towards some motion artifact resulting in artifactual signal loss (note that we were never able to observe this artifactual attenuation on phantoms). However,

standard deviation of individual scans phase, which is a marker of translational motion, was not higher than usual ($\approx 10^\circ$) for these experiments. Hence it was not possible to discard corrupted data using an objective criterion based on phase standard deviation. Ideally, we would need an internal standard of known diffusivity that would allow us to retrospectively discard datasets where signal attenuation for this internal standard is stronger than expected. Unfortunately, water cannot be used for this purpose, because its ADC depends on the tissue composition and on the diffusion time *in vivo*.

In contrast, MM are large molecules with very low diffusion coefficient, as shown in the precedent part. When using a moderate b such as used in the present study, we expect their signal to remain almost constant. MM signal exhibiting stronger attenuation than expected can be considered as an objective criterion to detect motion artifact and to discard data. The isolated MM peak at 0.9 ppm is still visible at TE=60 ms, and is the only one that does not overlap with metabolites, making it suitable to reliably quantify MM signal attenuation. Let's introduce the factor M to quantify this attenuation. It is defined as the ratio between the MM signal on diffusion-weighted spectra (S_{DW}) and on the spectrum at $b=0$ ms/ μm^2 (S_0) for each data set, integrated over the 0.8-1.0 ppm region, spectra being pre-processed to have the same phase and frequency (as determined on metabolite signal) and same vertical offset (whose value is determined by signal averaging just around the MM peak, i.e. from 0.77 to 0.8 ppm and from 1 to 1.03 ppm):

$$M = \frac{\int_{0.8}^{1.0} S_{DW} dppm}{\int_{0.8}^{1.0} S_0 dppm} \quad (4)$$

In the above section about signal attenuation at high b , MM ADC appeared fairly stable at 35 and 99 Hz with an average value of $0.026 \mu\text{m}^2/\text{ms}$, and in the following we will assume that this remains the case at higher frequencies, which is the most conservative assumption to avoid selection of datasets biased by motion artifact. To get a proper estimation of the expected MM signal attenuation at $b_{\text{max}}=1.2$ ms/ μm^2 we calculated the attenuation from

$\text{ADC}_{\text{MM}}=0.026 \mu\text{m}^2/\text{ms}$, giving $M_{\text{expected}} = \frac{S}{S_0} = \exp(-0.026 \times 1.2) = 0.97$. Sometimes M is slightly larger than 1, which corresponds to measurement noise. We propose to retrospectively evaluate the noise by taking the difference between M_{expected} and the maximal M over all acquired spectra in all datasets, which is found to be 1.03, corresponding to a noise of 0.06 (1.03 minus 0.97). In the end, we decide to only retain datasets for which M is comprised in $M_{\text{expected}} \pm \text{noise}$, corresponding to M in the 0.91-1.03 range.

As can be seen in Figure 4, many acquisitions at high frequencies fell outside the $M_{\text{expected}} \pm \text{noise}$ window, and were consequently discarded. More specifically, rejection rate was 0% at 35 Hz and 3% (1/29) at 99 Hz, but for other frequencies rejection rate was very high (70% at 150 Hz, 56% at 201 Hz, and 50% at 252 Hz) (see Table 1).

In Figure 5, ADC for the five metabolites (NAA, tCr, tCho, Ins and Tau) are displayed as a function of the diffusion frequency, after dataset sorting. Error bars are bigger at high

frequencies and especially at $\omega_{eff}=150$ Hz and $\omega_{eff}=201$ Hz, which is mainly due to the corresponding high rejection rate and consequently the smaller number of valid acquisitions. ADC at 35 Hz and 99 Hz appear higher than in the previous section when they were determined by log-linear regression up to $b=8$ ms/ μm^2 , simply because attenuation is not-strictly mono-exponential so that going to higher b leads to ADC underestimation. Overall, these data point towards a general tendency of ADC to increase with frequency, consistently with less and less hindered diffusion, and metabolites approaching free diffusion. Since ADC values for metabolites *in vivo* at 252 Hz are about one third of their free diffusion coefficient, cytosol viscosity can be estimated to be less than three times pure water viscosity.

Discussion

Interest of stretched gradients

The stretched cosines as introduced here constitute a class of oscillating waveforms connecting two previously used waveforms. The $\alpha=1$ case corresponds to the apodized cosine (10). When α tends to infinite, the stretched cosine approaches the “trapezoid-cosine” waveform used by Van et al. (13) (but in our case we assume an infinitely short rise time, so that $\alpha=\infty$ represents an ideal situation which can never be reached in practice). When α varies between these two extremes, stretched cosines allow a flexible way to increase b while preserving acceptable gradient modulation spectrum, where the stretching exponent α can be chosen depending on gradient’s maximal slew rate and performance in terms of eddy currents, or on the maximal acceptable contamination of secondary lobes on the gradient modulation spectrum. This is for example particularly striking when considering the secondary lobes just above 400 Hz in Figure 1C, where $\alpha=4$ and ∞ lead to $\sim$ doubled contamination compared to $\alpha=2$. There is some similarity between stretched gradients parameterized by α , ranging from $\alpha=1$ to infinity, and stretched hyperbolic secant radiofrequency pulses parameterized by n , ranging from $n=1$ (regular hyperbolic secant) to infinity (CHIRP), which also allow flexible compromise between maximal amplitude of the radiofrequency field B1 and adequate frequency selection (19).

Recent studies (13,20,21) report the use of oscillating gradients to explore microstructure at short scales in a clinical setting. However, OG experiments on clinical scanners remain very challenging, due to the limited maximal gradient strength. A few strategies have been proposed to optimize diffusion-weighting. The “trapezoid-cosine” waveform (13) can indeed be well optimized in terms of diffusion-weighting and frequency selection but is very constraining for the gradient if used at gradient’s maximal capacities (rise time and strength). Lundell et al. (21) have introduced circularly polarized oscillating gradients, which yield two-fold increased b compared to single-direction oscillating gradient, but cannot be adapted to diffusion-weighting along the three axes simultaneously. Stretched cosines may be interesting in a clinical context and complement these existing approaches.

In this work, we have taken advantage of the higher diffusion-weighting induced by stretched gradients to reduce TE down to 60 ms, which allowed the detection of a higher number of metabolites compared to our previous study performed at TE=154 ms in the rat brain, where only NAA, tCr and tCho were observed (6). More importantly maybe, shorter

TE allowed the quantification of the macromolecule peak at 0.9 ppm. This peak could subsequently be used as an objective criterion to detect and discard datasets corrupted by motion artifacts.

Motion artifact associated with oscillating gradient

The observation of an intermittent artifact was much unexpected, since we have never observed such artifact in our past works, at least when MM signal was observable. This was generally the case in our preclinical studies using pulsed field gradients (PFG), although at diffusion times longer than 1 second, it was generally not possible to reliably quantify MM signal. Interestingly, some particular gradient frequencies seem to be more susceptible to this artifact than other frequencies (number of acquisitions rejected depending on the frequency: 0/29 at 35 Hz, 1/29 at 99 Hz, 21/30 at 150 Hz, 15/27 at 201 Hz, 15/30 at 252 Hz). The high rejection rate at some frequencies reduces the number of valid datasets, unfortunately increasing the standard error of the mean for the ADC at these frequencies, and imposing a very large number of experiments to retain enough usable datasets.

Generally speaking, we don't think that motion artifacts observed here can be related to any physiological process such as cardiac pulsation or respiration, because problematic frequencies don't correspond to any physiological frequency (mouse respiration rate under isoflurane is ~1-2 Hz, mouse heart beating rate is ~8-10 Hz). Even brain action potentials are too slow (maximum ~50 Hz). Nevertheless, to assess whether this artifact could be mitigated using respiratory gating, we actually performed an additional experiment at ω_{eff} =150, 201 and 252 Hz with respiratory gating. It turned out that respiratory gating had no effect, as we still detected a strong artifactual signal attenuation at ω_{eff} =201 Hz and no artifact at other frequencies. Furthermore, we wanted to verify in one *in vivo* experiment that these artifacts were not, for some reason, related to the special gradient waveforms used here. It turned out that, in this experiment, artifactual signal drop also occurred at the same problematic frequency (201 Hz) when using standard apodized cosine.

In our opinion, the artifact observed here is related to the special nature of OG in comparison with PFG. When using OG, signal attenuation depends on the motion harmonics at the corresponding gradient frequency. In the meantime, gradient oscillation can potentially continuously drive mechanical vibrations at the same frequency (which is precisely the one probed by the measurement). In contrast, for PFG the potential mechanical vibrations during the diffusion time are not correlated with the diffusion time (for the simple reason that the mechanical vibrations during t_d can only be caused by the first gradient pulse). Although a previous study has reported motion artifact resulting in increased ADC (and larger error bars) at 150 Hz, including on phantom (22), in our case gradient vibrations caused by OG are certainly moderate, since we never observed artifactual signal attenuation on phantoms. However, it is perfectly conceivable that the brain enters in mechanical resonance for some of these vibration frequencies, which would ultimately results in motion artifact.

The pioneer aspect of our work makes comparison with literature quite difficult. However, we can compare current results with data acquired in the rat brain at 7 T, using an equivalent OG sequence (6). It turns out that ADCs at equivalent frequencies are 20-30% higher in this

past study. Species are different but we think this is unlikely to explain this difference. More importantly, macromolecules were invisible because TE was longer in the rat brain study (154 ms), so the presence of potential motion artifact cannot be ruled out in this past study, which might partly explain the higher metabolite ADC.

Estimation of tissue microstructure

From recent studies at very long diffusion times in rodent, primate and human brain (7–9) showing that metabolite ADC is very stable, we have concluded that metabolites are not significantly confined within organelles or subcellular domains such as cell bodies, and are instead primarily diffusing along fibers (axons, dendrites, astrocytic processes etc). In that context, the variation of metabolite ADC at high frequencies may be mainly interpreted and modeled in terms of restriction in the transverse plane of fibers, as also done for water (23,24). This can be achieved using a model of impermeable, infinite (valid for short times) and randomly oriented (valid in first approximation in a large voxel with fibers in all orientations) cylinders, which is referred to as the “neurite model” in ref. (6,8)). In ref. (6) we had applied such a model on metabolite ADC at high frequencies, using Callaghan and Stepisnik’s formalism of velocity autocorrelation function in the frequency domain to calculate the diffusion spectrum $D(\omega)$ (see Theory section and Eq. (2)), which is adapted to oscillating gradients at low b-values where the phase distribution can be considered Gaussian (15,25,26). For simplicity we assumed a very sharp gradient modulation spectrum without secondary lobes, resulting in a perfectly clean sampling of the diffusion spectrum, i.e. $ADC = D(\omega_{eff})$. A more exact calculation could be desirable for more accurate modeling, e.g. as performed for finite sinusoidal gradients (27), or by numerical integration of $D(\omega) \times |F(\omega)|^2$, where $F(\omega)$ could be numerically computed for the actual gradient shapes, but the goal here was to use a simple approach to get a estimate of typical radii and intracellular diffusivities. This yielded typical radii of $\sim 0.9 \mu m$ and typical intracellular diffusivities $D_{intra} \sim 0.5\text{--}0.6 \mu m^2/ms$, corresponding to a low-viscosity cytosol ($\sim 25\%$ higher compared to pure water).

How are the present data, where dataset sorting was performed based on MM attenuation criterion, still consistent with the neurite model? The best fits obtained with the model (see (6) for mathematical details about the modeling) are shown in Figure 5, and the radius and D_{intra} for each metabolite are reported in Table 2. Standard deviations were estimated using Monte-Carlo simulations (1000 repetitions). Very interestingly, it appears that the model actually yields values which are not very different from ref. (6), yielding radii in the $0.8\text{--}1.0 \mu m$ range, and D_{intra} corresponding to a low-viscosity cytosol ($\sim 20\text{--}30\%$ higher viscosity compared to pure water). Note that, if datasets corrupted by motion artifacts are not discarded, radii are estimated to be in the $1.6\text{--}1.9 \mu m$ range, i.e. approximately doubled, while D_{intra} remain essentially unchanged. However it should be kept in mind that the potential bias on estimated parameters induced by motion artifact may be different in different situations, depending on the relative importance of the artifact at lower or higher frequencies.

It should also be noted that measured ADC are still more than three times lower than estimated D_{intra} even at 252 Hz, so diffusion can certainly not be considered as free yet. This

makes the estimation of cytosol viscosity rely a lot on the modeling. To get a more accurate estimation of D_{intra} , reaching significantly higher frequencies would be required, e.g. using a stronger gradient coil, to experimentally measure metabolite ADC as close as possible to D_{intra} . In our opinion, the measurement of D_{intra} may be interesting beyond fundamental knowledge, because it is conceivable that massive accumulation of proteins may increase cytosol viscosity / molecular crowding in the context of proteopathies such as Alzheimer's or Huntington's disease.

Conclusion

In the present work, we report a strategy to perform *in vivo* DW-MRS measurements on metabolites at ultra-short diffusion times. The new stretched cosine waveforms allow increasing diffusion-weighting by more than 50% or, conversely, reducing TE. Reducing TE makes the signal of some J-coupled metabolites and macromolecules observable, thus providing a larger number of molecular probes to explore the intracellular space. Observing macromolecules actually proves crucial because, using macromolecules as an internal reference of approximately null diffusivity, a quality control criterion can be proposed to discriminate unbiased ADC measurements from ADC measurements biased by some motion artifact. The methods proposed here should prove useful to probe short-range intracellular structure and viscosity using oscillating gradients DW-MRS. Finally, although the motion artifact reported here (strong artifactual signal attenuation in the absence of scan-to-scan phase variation) seems to be associated with OG, we think it might be good practice to quantify MM signal and check the absence of motion artifact when performing PFG experiments, at least when low b values are used so that MM signal is expected to remain fairly stable.

Acknowledgements

This work was supported by the European Research Council ("INCELL" project, ERC grant 336331). The 11.7 T MRI scanner was funded by a grant from "Investissements d'Avenir - ANR-11-INBS-0011" - NeurATRIS: A Translational Research Infrastructure for Biotherapies in Neurosciences.

Abbreviations

ADC	apparent diffusion coefficient
CRLB	Cramér-Rao lower bounds
DW-MRS	diffusion-weighted magnetic resonance spectroscopy
Ins	myo-inositol
MM	macromolecules
NAA	N-acetyl-aspartate
OG	oscillating gradients
PFG	pulsed field gradients

SNR	signal-to-noise ratio
Tau	taurine
tCho	choline compounds
tCr	total creatine
td	diffusion time
TE	echo time

Appendix

To ensure that there is no major contamination in the gradient modulation spectrum $F(\omega)$, we can investigate the worst-case scenario of an oscillating crenel corresponding to alpha infinite. The integral of an infinite oscillating crenel at frequency ω is an oscillating triangle $T(t)$. The corresponding Fourier series of this periodic function is:

$$T(t) = \frac{8}{\pi^2} \sum_{k=0}^{\infty} (-1)^k \frac{\sin((2k+1)\omega t)}{(2k+1)^2} \quad (\text{A1})$$

The gradient modulation spectrum is easily found to be a series of Dirac functions with rapidly decaying amplitude at increasing frequencies:

$$F(\omega) = FT(T(t)) = \frac{8}{\pi^2} (\delta(\omega) + \frac{1}{9} \delta(3\omega) + \frac{1}{25} \delta(5\omega) \dots) \quad (\text{A2})$$

The square of the gradient modulation spectrum, which determines the contribution of the various frequencies of the diffusion spectrum to the signal attenuation (see Eq. (2)), is immediately determined:

$$F(\omega)^2 = \frac{8}{\pi^2} (\delta(\omega) + \frac{1}{81} \delta(3\omega) + \frac{1}{625} \delta(5\omega) \dots) \quad (\text{A3})$$

It appears that the contamination from higher frequencies is very limited, e.g. the corresponding terms are weighted in 1/81 for 3ω , 1/625 for 5ω , etc... in comparison with the main frequency ω .

Note that, in realistic situations, the gradient waveform is not infinite, so that secondary lobes with potentially larger amplitude appear.

References

1. Nicolay K, Braun KP, Graaf RA, Dijkhuizen RM, Kruiskamp MJ. Diffusion NMR spectroscopy. NMR in biomedicine. 2001; 14(2):94–111. [PubMed: 11320536]

2. Ronen I, Valette J. Diffusion-weighted magnetic resonance spectroscopy. *eMagRes*. 2015; 4:733–750.
3. Cao P, Wu EX. In vivo diffusion MRS investigation of non-water molecules in biological tissues. *NMR in biomedicine*. 2016
4. Duong TQ, Sehny JV, Yablonskiy DA, Snider BJ, Ackerman JJ, Neil JJ. Extracellular apparent diffusion in rat brain. *Magnetic resonance in medicine*. 2001; 45(5):801–810. [PubMed: 11323806]
5. Branzoli F, Techawiboonwong A, Kan H, Webb A, Ronen I. Functional diffusion-weighted magnetic resonance spectroscopy of the human primary visual cortex at 7 T. *Magnetic resonance in medicine*. 2013; 69(2):303–309. [PubMed: 23165888]
6. Marchadour C, Brouillet E, Hantraye P, Lebon V, Valette J. Anomalous diffusion of brain metabolites evidenced by diffusion-weighted magnetic resonance spectroscopy in vivo. *Journal of cerebral blood flow and metabolism*. 2012; 32(12):2153–2160. [PubMed: 22929443]
7. Palombo M, Ligneul C, Najac C, Le Douce J, Flament J, Escartin C, Hantraye P, Brouillet E, Bonvento G, Valette J. New paradigm to assess brain cell morphology by diffusion-weighted MR spectroscopy in vivo. *Proceedings of the National Academy of Sciences of the United States of America*. 2016; 113(24):6671–6676. [PubMed: 27226303]
8. Najac C, Marchadour C, Guillermier M, Houitte D, Slavov V, Brouillet E, Hantraye P, Lebon V, Valette J. Intracellular metabolites in the primate brain are primarily localized in long fibers rather than in cell bodies, as shown by diffusion-weighted magnetic resonance spectroscopy. *NeuroImage*. 2014; 90:374–380. [PubMed: 24382523]
9. Najac C, Branzoli F, Ronen I, Valette J. Brain intracellular metabolites are freely diffusing along cell fibers in grey and white matter, as measured by diffusion-weighted MR spectroscopy in the human brain at 7 T. *Brain structure & function*. 2016; 221(3):1245–1254. [PubMed: 25520054]
10. Does MD, Parsons EC, Gore JC. Oscillating gradient measurements of water diffusion in normal and globally ischemic rat brain. *Magnetic resonance in medicine*. 2003; 49(2):206–215. [PubMed: 12541239]
11. Parsons EC Jr, Does MD, Gore JC. Temporal diffusion spectroscopy: theory and implementation in restricted systems using oscillating gradients. *Magnetic resonance in medicine*. 2006; 55(1):75–84. [PubMed: 16342147]
12. 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 in biomedicine*. 2010; 23(7):745–756. [PubMed: 20677208]
13. Van AT, Holdsworth SJ, Bammer R. In vivo investigation of restricted diffusion in the human brain with optimized oscillating diffusion gradient encoding. *Magnetic resonance in medicine*. 2014; 71(1):83–94. [PubMed: 23447055]
14. Stepisnik J. Analysis of Nmr Self-Diffusion Measurements by a Density-Matrix Calculation. *Physica B & C*. 1981; 104(3):350–364.
15. Callaghan PT, Stepisnik J. Frequency-Domain Analysis of Spin Motion Using Modulated-Gradient Nmr. *J Magn Reson Ser A*. 1995; 117(1):118–122.
16. Provencher SW. Estimation of metabolite concentrations from localized in vivo proton NMR spectra. *Magnetic resonance in medicine*. 1993; 30(6):672–679. [PubMed: 8139448]
17. Pfeuffer J, Tkac I, Gruetter R. Extracellular-intracellular distribution of glucose and lactate in the rat brain assessed noninvasively by diffusion-weighted ¹H nuclear magnetic resonance spectroscopy in vivo. *Journal of cerebral blood flow and metabolism*. 2000; 20(4):736–746. [PubMed: 10779018]
18. Ligneul C, Palombo M, Valette J. Metabolite diffusion up to very high b in the mouse brain in vivo: Revisiting the potential correlation between relaxation and diffusion properties. *Magnetic resonance in medicine*. 2016; doi: 10.1002/mrm.26217
19. Garwood M, DelaBarre L. The return of the frequency sweep: designing adiabatic pulses for contemporary NMR. *Journal of magnetic resonance*. 2001; 153(2):155–177. [PubMed: 11740891]
20. Baron CA, Beaulieu C. Oscillating gradient spin-echo (OGSE) diffusion tensor imaging of the human brain. *Magnetic resonance in medicine*. 2014; 72(3):726–736. [PubMed: 24142863]

21. Lundell H, Sonderby CK, Dyrby TB. Diffusion weighted imaging with circularly polarized oscillating gradients. *Magnetic resonance in medicine*. 2015; 73(3):1171–1176. [PubMed: 24639209]
22. Xu JZ, Li K, Smith RA, Waterton JC, Zhao P, Chen HD, Does MD, Manning HC, Gore JC. Characterizing Tumor Response to Chemotherapy at Various Length Scales Using Temporal Diffusion Spectroscopy. *PloS one*. 2012; 7(7)
23. Xu J, Li H, Harkins KD, Jiang X, Xie J, Kang H, Does MD, Gore JC. Mapping mean axon diameter and axonal volume fraction by MRI using temporal diffusion spectroscopy. *NeuroImage*. 2014; 103:10–19. [PubMed: 25225002]
24. Li H, Jiang X, Wang F, Xu J, Gore JC. Structural information revealed by the dispersion of ADC with frequency. *Magnetic resonance imaging*. 2015; 33(9):1083–1090. [PubMed: 26117695]
25. Stepisnik J, Mohoric A, Duh A. Diffusion and flow in a porous structure by the gradient spin echo spectral analysis. *Physica B-Condensed Matter*. 2001; 307(1–4):158–168.
26. Stepisnik J. Time-Dependent Self-Diffusion by Nmr Spin-Echo. *Physica B-Condensed Matter*. 1993; 183(4):343–350.
27. Xu J, Does MD, Gore JC. Quantitative characterization of tissue microstructure with temporal diffusion spectroscopy. *Journal of magnetic resonance*. 2009; 200(2):189–197. [PubMed: 19616979]

A. Gradient shape **B. Normalized $|F(\omega)|^2$** **C. Zoom on normalized $|F(\omega)|^2$** **D. Gain in b** **Figure 1.**

Comparison of stretched cosine waveforms for $\alpha=1$ (usual apodized cosine), 2, 4 and infinite. (A) Gradient waveforms; (B) Corresponding squares of gradient modulation spectra, $|F(\omega)|^2$, normalized by their integral to facilitate comparison; (C) Zoom on $|F(\omega)|^2$ to visualize secondary lobes; (D) Gain in b-value as a function of α for a given maximal gradient strength.

Figure 2.

(A) Typical stack plots acquired at $\omega_{eff}=35\text{ Hz}$ and $\omega_{eff}=99\text{ Hz}$ in a single experimental session. All spectra were acquired in a large $6\times 2.4\times 5\text{ mm}^3$ voxel in the mouse brain. No line broadening was applied; (B) Logarithm of signal attenuation for each metabolite and the macromolecules at two different frequencies ($\omega_{eff}=35\text{ Hz}$ and $\omega_{eff}=99\text{ Hz}$). Error bars stand for the standard deviation determined on four mice. Error bars for macromolecules are too large to be represented.

Figure 3.

Spectra acquired during the same experimental session from the same dataset, at $b=0$ and at $b=1.2$ ms/ μm^2 and at two different diffusion frequencies (spectrum at $b=0$ ms/ μm^2 is the same). (A) Spectrum with a normal signal macromolecule attenuation (satisfactory macromolecule factor, $M=0.98$); (B) Spectrum with a very strong macromolecule signal attenuation ($M=0.40$).

Figure 4.

Macromolecule attenuation factors calculated for each dataset on mice. The red line corresponds to the expected macromolecule attenuation $M_{expected}=0.97$, and the grey lines delimit the range of reliable data sets ($M_{expected} \pm noise$, corresponding to the 0.91-1.03 range). See Table 1 for the number of scans acquired at each frequency.

Figure 5.

Averaged ADC for NAA, tCr, tCho, Ins and Tau as a function of the frequency, after dataset sorting based on the MM attenuation criterion. Error bars stand for the standard deviation for these points. Blue line represents the best fit from neurite model for each metabolite averaged dataset.

Table 1

Number of scans acquired at each frequency (see Methods), and number of scans rejected based on MM signal attenuation (see Results and Discussion).

Frequency ω_{eff} (in Hz)	Number of acquired scans	Number of rejected scans
35	29	0
99	29	1
150	30	21
201	27	15
252	30	15

Table 2

Parameters determined from neurite model for each metabolite.

	$D_{\text{intra}} (\mu\text{m}^2/\text{ms})$	Radius (μm)
NAA	0.50 ± 0.02	0.78 ± 0.03
tCr	0.65 ± 0.04	0.79 ± 0.05
tCho	0.54 ± 0.06	0.82 ± 0.09
Ins	0.62 ± 0.01	0.82 ± 0.01
Tau	0.88 ± 0.15	1.04 ± 0.45