Transport mechanisms of intracellular metabolites in the brain: new insights by diffusion-weighted NMR spectroscopy with oscillating gradients

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Introduction:
Transport of molecules within cells is a key process in cellular biology. Transport can be achieved either passively by random diffusion, or by active transport, including specific molecular motors conveying cargos, or general convection of the cytosol (known as cytoplasmic streaming or cyclosis). The relative contribution of passive and active transport mechanisms to the apparent diffusion coefficient (ADC) of intracellular metabolites and intracellular water is still debated. As a matter of fact, many works have invoked failure of energy-dependent active transport, in particular cytoplasmic streaming, as a possible explanation for the observed massive ADC drop of brain intracellular molecules observed in ischemic stroke (eg. [1-4]). In the present work, we investigate metabolic motion in the rat brain in vivo using an original diffusion-weighted NMR spectroscopy (DW-MRS) approach using oscillating gradients. With this strategy, the temporal dependence of brain metabolite ADC is observed for the first time, for diffusion frequency \( a_0 \) ranging from 0 to 19 Hz to 267 Hz (corresponding to diffusion time \( t_D \) ranging from \(-1\) ms to \(-13\) ms). Exploiting the unique ability of oscillating gradients to probe the motion spectrum, it appears that random diffusion is the dominant transport mechanism for intracellular metabolites in the living brain. Armed with this knowledge, we perform data modeling based on geometrically constrained diffusion. Estimated parameters are consistent with known cellular architecture, further ruling out the plausibility of significant active transport.

Theory:
A powerful framework for the study molecular motion is the formalism of velocity autocorrelation function \( VAF(\omega) \) as given by Stepisnik [5]. The first model consisted in hollow cylinders isotropically oriented in 3D, to account for diffusion in long fibers ("neurite model"); unknown parameters were the free diffusion coefficient \( D_{free} \) and the fiber diameter \( d_f \). Second model consisted in interconnected spherical pores to account for diffusion in the tortuous internem of large cell bodies filled with organelles ("cell body model"); unknown parameters were \( D_{free} \), the pore diameter \( d_p \) and the tortuosity \( \tau \).

Results and discussion:
High quality spectra could be obtained, with eye-visible dependence of signal attenuation during single scanning sessions (Fig. 1B). At shorter \( a_0 \) (longer \( t_D \)), metabolite ADC is close to results already published for eddy currents on metabolite spectra. No phase variation was observed between individual scans, so spectra were simply summed before quantification by LCModel [7]. Finally, ADC was calculated for the most reliably quantified metabolites: N-acetylaspartate (NAA), total choline (tCho) and total creatine (tCr).

Diffusion models:
ADC averaged over the three metabolites was fitted using two models of restricted diffusion based on the theoretical expression of \( D(\omega) \) as given by Stepisnik [5]. The first model consisted in hollow cylinders isotropically oriented in 3D, to account for diffusion in long fibers ("neurite model"); unknown parameters were the free diffusion coefficient \( D_{free} \) and the fiber diameter \( d_f \). Second model consisted in interconnected spherical pores to account for diffusion in the tortuous internem of large cell bodies filled with organelles ("cell body model"); unknown parameters were \( D_{free} \), the pore diameter \( d_p \) and the tortuosity \( \tau \).