Diffusion-Weighted NMR Spectroscopy *In Vivo* to Probe Brain Cell Structure – and Beyond

Julien Valette[1]

[1]MIRCen, Commissariat à l'Energie Atomique et aux Energies Alternatives, Fontenay-aux-Roses, France

Diffusion-weighted NMR spectroscopy (DW-MRS) offers the unique ability to non-invasively quantify the diffusion of endogenous brain metabolites *in vivo* (1,2). In contrast to water, which is ubiquitous in biological tissues, most brain metabolites are confined within cells. Their diffusion properties are thus expected to mostly depend on intracellular parameters such as cytosol viscosity, molecular crowding, size and shape of the cell… Furthermore, some metabolites are thought to exhibit preferential compartmentation, with N-acetyl-aspartate (NAA) and glutamate (Glu) being mostly in neurons, while myo-inositol (mIns) and choline compounds (tCho) are thought to be preferentially compartmentalized in glial cells. Cell-specificity has been the main motivation driving methodological research and applications of DW-MRS *in vivo* over the last 25 years. Alterations of metabolite apparent diffusion coefficient (ADC) have been reported in brain diseases (e.g. (3,4)), but the origin of these variations remained unclear. The basic reason is that many potential factors may affect diffusion, the relative contribution of these various factors being not always clearly understood.

To elucidate what governs brain metabolite diffusion, my group engaged in the exploration of diffusion over very different diffusion times t_d, to probe intracellular space over very different spatial scales (reviewed in (5)). This revealed rapid decrease of ADC as t_d is increased, followed by a relatively stable plateau for long t_d, which is a very clear signature of diffusion along long and thin fibers. Moreover, these measurements ruled out any significant contribution of active transport or compartmentation in subcellular structure. This framework where diffusion measurements primarily reflect diffusion within long and thin cylinders is very consistent with "independent" experiments using high diffusion-weighting *b* or double-diffusion encoding (DDE) (reviewed in (6)). Such framework also lays foundations for new models to extract structural parameters, e.g. as recently proposed for ultra-long t_d, where the long-range structure of fibers was modeled with some branching and finite length (7), and where reconstructed compartments for glial metabolites (mIns, tCho) were found to be smaller and less complex than for neuronal metabolites (NAA, Glu), consistently with histology. Armed with such knowledge and methods, we tried to evaluate the potential of some of these approaches to quantitatively assess morphological variations under "pathological" conditions. We studied a mouse model of astrocyte reactivity, where astrocytes become hypertrophic. Among all intracellular metabolites, only mIns exhibited altered diffusion. Increased fiber diameter and fiber length were extracted for mIns cellular compartment by DW-MRS, consistently with astrocytic hypertrophy as quantitatively assessed by confocal microscopy (8). In the end, mIns is presumably highly specific to astrocytes, and DW-MRS appears to enable non-invasive quantification of morphologic alterations of specific brain cells.

Recently, we started exploring the capacity of DW-MRS to go beyond cell structure. Our interest originates from the measurement of lactate diffusion, and particularly its very strong variations during astrocyte reactivity (8). Unlike other metabolites, lactate is also extracellular, therefore its diffusion cannot be linked to cell structure in a "straightforward" way. Hence little attention was paid to its diffusion so far, despite its metabolic importance: lactate is thought to play a crucial in brain function, in particular *via* a hypothetic astrocyte-to-neuron lactate shuttle, which requires astrocytes to maintain larger lactate concentration than neurons. The distribution of lactate between the different cell types and the extracellular space remains a topic of intense debate, due to the lack of *in vivo* tools. We will try to convince the audience that DW-MRS, thanks to its ability to distinguish diffusion in different microstructural environments, may allow assessing brain lactate compartmentation. This requires well characterizing diffusion within astrocytes and neurons (e.g. based on diffusion measurement of cell-specific metabolites as described above) as well as extracellular diffusion. The latter is still poorly understood, but may be studied by DW-MRS of molecules injected in the extracellular space and remaining there, as already proposed in the past ((9) for review); we are currently revisiting such a strategy over a much broader variety of diffusion experiments (long t_d, high *b* and DDE) using injected sucrose.

In conclusion, basic understanding of brain metabolite diffusion has progressed over the last years, allowing relevant interpretation of DW-MRS in terms of cellular structure, thus opening new possibilities for microstructure quantification under normal and pathological conditions. Furthermore, DW-MRS may allow characterizing extracellular diffusion properties or metabolic compartmentation, thus going beyond cell structure determination.

References: (1) Nicolay K et al., *NMR Biomed* 2001;14(2):94-111. (2) Ronen I & Valette J, *eMagRes* 2015;4:733–750. (3) Dijkhuizen RM et al., *JCBFM* 1999;19(3):341-349. (4) Ercan E et al., *Brain* 2016;139(5):1447-1457. (5) Valette J et al., *Front. Neuroscience* 2018;12:2. (6) Palombo M et al., *NeuroImage* 2018;182:97-116. (7) Palombo M et al., *PNAS* 2016;113(24):6671-6676. (8) Ligneul C et al., *NeuroImage* 2019. (9) Kroenke CD & Neil JJ., *Neurochem Int* 2004;45(4):561-568.