Diffusion Tensor Spectroscopy: a promising tool for quantitative assessment of neuronal dysfunction ?

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Introduction

It has been largely demonstrated for years that severe brain damage like ischemia or stroke result in alteration of several indexes measured by NMR. Beyond classical changes in metabolites concentration, a global decrease of water ADC [1;2] and concomitant alteration of intracellular metabolites ADC have also been reported [3]. However diffusivity alterations have never been reported in the brain undergoing mild and partly reversible neuronal dysfunction. In such a situation DW-spectroscopy might reveal particularly interesting because of its ability to probe specific cell types, allowing to measure small variations of diffusivity within neurons while global indexes might remain unchanged. In this context measurement accuracy and reproducibility are critical, but may be degraded since measured ADC strongly depends on the orientation of anisotropic tissues (fibers…) relative to the diffusion gradient. Increased precision may be obtained by the use of diffusion tensor and the derivation of rotationally invariant indexes such as mean diffusivity *Dav* and fractional anisotropy FA. Our purpose was (i) to implement diffusion tensor spectroscopy (DTS) on a 3T whole body system, (ii) to acquire diffusion tensor for NAA, glutamate, creatine and choline in the monkey brain *in vivo* and (iii) to investigate changes in metabolite mean diffusivity induced by oxidative stress (chronic 3NP treatment).

Materials and Methods

Animal preparation: Two groups of animals were studied. For the 1st group (control), 5 experiments were performed on 2 healthy macaque monkeys (*macaca fascicularis*). Monkeys were anesthetized by an i.v. propofol infusion and ventilated. Physiological parameters were monitored and remained stable within a normal range. The 2d group was made of 3 macaque undergoing chronic oxidative stress induced by 3NP (a mitochondrial toxin largely used as a model for Huntington disease). 3NP treatment consisted in a daily injection (~30mg/kg) of the toxin over 12 months performed on 3 macaques. NMR measurement was performed 2 times on each treated macaques, resulting in a total of 6 experiments.

NMR acquisition: Experiments were performed on a whole-body 3T system (Bruker, Ettlingen, Germany). A surface coil was placed on top of the monkey head. A 4.5mL voxel was positioned in the fronto-parietal lobe. The diffusion-weighted sequence consisted in a modified STEAM sequence with TE/TM=21/110ms [4]. An inversion-recovery experiment was performed so that the resulting macro-molecule and lipid contribution could be subtracted from DW-spectra. Diffusion-weighted spectra (TR=2.5s, 192 transients) were acquired at 3 different *b* values (0, 500 and 1000 s/mm²) along 6 directions. To correct for small movement artifact, scan-to-scan phasing was performed. Spectra were analyzed with LCModel [5]. Data were automatically processed for diffusion tensor calculation, and then *Dav* (trace of the tensor divided by 3) and FA were derived by home-made routines.

Results and Discussion

Control monkeys: Fig.1 shows a stack plot of DW-spectra acquired along one direction. For the control group *Dav* was 0.125±0.017µm²/ms for NAA, 0.189±0.013µm²/ms for glutamate, 0.085±0.022µm²/ms for creatine, 0.083±0.033µm²/ms for choline. FA was 0.66±0.03 for NAA, 0.75±0.08 for glutamate, 0.78±0.17 for creatine and 0.77±0.03 for choline. As far as we know, the only other work reporting metabolites *Dav* was performed in the rat brain at 4.7T [6], although diffusion tensor was not effectively acquired. Mean diffusivity was typically 50% higher in that study, which can be ascribed to the shorter diffusion time (18.5ms) which softens restriction effects. Metabolite diffusion cannot be considered isotropic in the VOI as indicated by the high FA (mean for all metabolites: 0.75±0.05). More precisely diffusion mainly occurs in the coronal XY plane, while diffusion along the Z axis is limited. This result is consistent with DTI studies in the corresponding region of the human brain.

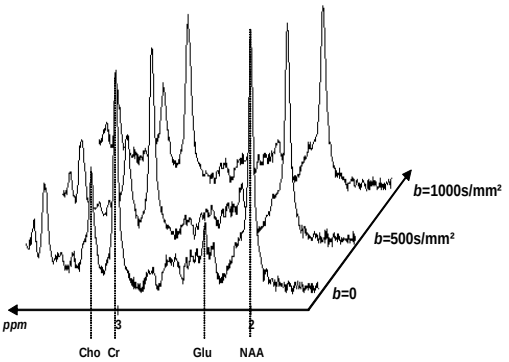

Fig.1: stack plot of DW spectra along the XZ direction (NS=192, no filtering)

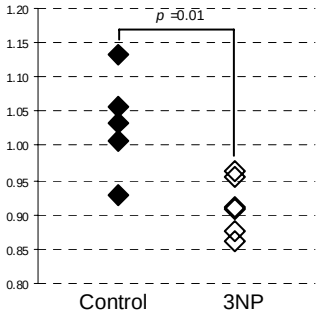

Fig.2: ratio *Dav*(NAA)/mean(*Dav*) for control and 3NP groups

3NP treated monkeys: Dav was 0.106±0.009µm²/ms for NAA, 0.204±0.025µm²/ms for glutamate, 0.088±0.015µm²/ms for creatine, 0.066±0.023µm²/ms for choline (FA were respectively 0.72±0.10, 0.67±0.16, 0.75±0.14 and 0.80±0.06). For each metabolite, statistical difference between the two groups was tested on *Dav* and FA. No significant difference was found except for the mean diffusivity of NAA which undergoes a 15% highly significant decrease under 3NP treatment (p=0.03, non-parametric Wilcoxon rank sum test). Note that conventional ADC measurements (single gradient direction) were also performed but failed to detect any change under 3NP treatment due to the larger dispersion of ADC values. This demonstrates the advantage of DTS upon conventional ADC measurement to detect mild alteration of diffusivity.

In contrast, no MRI lesion was visible within the VOI and lactate concentration, NAA/Cr ratio and water *Dav* did not change. However metabolic dysfunction was clearly assessed. Indeed the brain TCA cycle flux has been measured by ^{13}C NMR spectroscopy on treated monkeys, revealing a 40% decrease of oxidative metabolism associated with 3NP treatment (data not shown). The fact that the diffusivity of other metabolites was not altered could be explained either by the higher accuracy on NAA measurement or by a specific decrease of NAA diffusivity. In order to investigate this point, the inter-individual variability due to physiological/structural parameters was limited by calculating the normalized ratio *Dav*(NAA)/mean(*Dav*) for each single experiment (mean(*Dav*) is the average of *Dav* for the 4 metabolites). For control monkeys, the ratio is always greater than 1 (1.04±0.07) except for one experiment, whereas it is always smaller than 1 for the 3NP group (0.91±0.04) (see fig. 2). The separation between both groups is indeed improved (p=0.01). This argues in favour of NAA diffusion being more affected by 3NP treatment than other metabolites, probably because of the neuronal specificity of NAA. Note that a similar effect on neuron-compartmentalized glutamate was not detected, most likely due to the lower accuracy of glutamate quantitation.

Conclusion

As far as we know, this work is the first reported DTS study of brain metabolites *in vivo*. Diffusion tensor allowed to obtain highly reproducible measurements of metabolites mean diffusivity in the monkey brain despite the high anisotropy of brain tissue. DTS accuracy revealed critical for detecting a significant 15% decrease of NAA mean diffusivity under oxidative stress while other indexes (water diffusion, lactate and NAA concentration) did not exhibit significant change. This study argues in favor of DTS being a promising tool for assessing neuronal dysfunction and potential reversal by neuroprotective strategies.

References

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