Neurochemical profiling of two rodent Parkinson's disease models: an *in vivo* MR spectroscopy study

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INTRODUCTION

Animal models of Parkinson's disease (PD) provide an experimental means to define early markers of pathological changes that may affect the nigrostriatal pathway prior to overt neurodegeneration. In this study we investigated the variations that may occur in striatal metabolite concentrations by MR spectroscopy (MRS). We compared neurochemical profiles in two animal models of PD. The first model is based on the unilateral injection of 6-hydroxydopamine (6-OHDA), a toxin which selectively affects dopaminergic neurons. In the second model, we injected an adeno-associated viral vector (AAV) encoding human alpha-synuclein (aSyn), a protein involved in familial as well as sporadic cases of PD, in the *substantia nigra* [1]. In both models, the non-injected contralateral hemisphere provided a control value for metabolite concentrations in healthy conditions. We used the 6-OHDA model to establish the differences that are due to extensive striatal denervation and then determined which of these changes may appeared in the AAV-aSyn model, expected to more faithfully replicate early pathological alterations leading to PD in humans. Although toxin-based PD animal models have already been investigated by MRS, only a few studies have been reported [2, 3]. Moreover, the use of an ultrahigh-field 14.1 T MR magnet for the *in vivo* assessment of neurochemical profiles allowed for the sensitive detection of 21 brain metabolites, which has only been attained in very few animal studies yet. Finally, the use of two different animal models provided a comparison to detect markers of the proper pathology.

METHODS

6-OHDA model: Six female Sprague-Dawley rats (180-200g) were stereotaxically injected with 4 µl of a 5mg/ml solution of 6-OHDA in ascorbic-saline in the medial forebrain bundle of the right hemisphere. MRS studies were performed one week before and three weeks after injection on a horizontal 14.1 T magnet (Varian/Magnex) using a custom-built quadrature coil. After shimming was performed using FASTMAP, localized proton spectra (27 µl) were acquired in the striatum of the right hemispheres using SPECIAL [4] with 240 averages, TE = 2.8 ms and TR = 4 s (Fig. 1). As controls, spectra were also acquired in the striatum of the left non-injected hemisphere. Absolute quantification of 21 metabolites of the neurochemical profile was performed using LCModel.

aSyn overexpression model: Five female Sprague-Dawley rats were stereotaxically injected with 2.8$^{\text{E}}$7 transducing units of AAV2/6 vectors coding for human α-synuclein [1] in the *substantia nigra pars compacta* of the right hemisphere. A control group of six rats was injected with non-coding AAV2/6 vectors. NMR measurements were performed 12 weeks after injection according to the same protocol as for the 6-OHDA model.

Figure 1: Axial MR image of the rat brain showing the voxel position for localized spectroscopy of the striatum.

In order to quantify significant changes caused in the striatum by the lesions, the metabolite concentration ratios between the two brain hemispheres were calculated for each animal. Significance in the changes between both hemispheres in each animal was then determined using the paired two-tailed student T-test.

RESULTS AND DISCUSSION

After shimming, the average linewidth was 17 Hz and the SNR was ~30 (Fig. 2). Most metabolites were quantified with Cramer-Rao lower bounds lower than 10% (15% for the five lowest concentration metabolites). Significant changes in metabolite concentration are reported here for γ-aminobutyric acid (GABA), N-acetyl aspartate (NAA) and glutamate (Glu) (Table 1).

After 6-OHDA injections, the following significant metabolite changes were observed in the injected hemisphere compared to the control hemisphere: 20% increase of GABA, 7% decrease of NAA and 6% decrease of glutamate. These results are in good agreement with previous studies performed on various toxin-based PD animal models [2, 5-7], except for glutamate, for which various effects have previously been reported [3, 6]. In contrast, we did not observe any significant difference in concentrations of the metabolites assessed here between both hemispheres prior to 6-OHDA injections, confirming that there was no initial brain asymmetry in their distribution. Interestingly, measurements performed on the aSyn overexpression model also showed a significant increase of the GABA level (12%) in the striatum of the affected hemisphere. No other significant metabolite change was detected in this model. Moreover, the control group injected with a non-coding vector did not show any significant metabolite change, demonstrating that the observed effects are indeed due to aSyn expression.

To our knowledge, this study is the first spectroscopic analysis performed on an animal model based on aSyn overexpression. Based on the comparison between these two models of PD, we conclude that the GABA increase observed in the striatum of the lesioned hemisphere reflects early pathological alterations and might constitute a sensitive marker of the disease process.
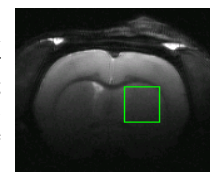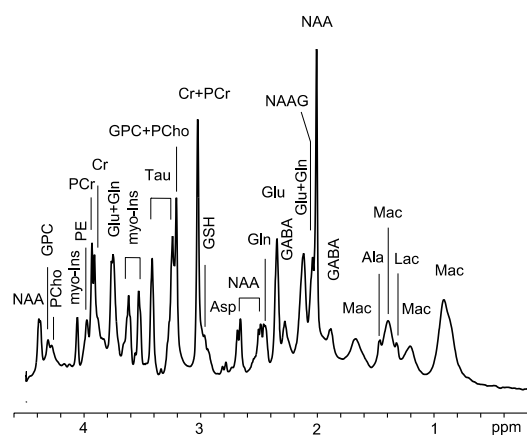

Figure 2: Representative localized spectrum of the right hemisphere 3 weeks after 6OHDA injection. In addition to the metabolites represented on the spectrum, Gly, Scyllo, Asc and Glc were also quantified.

	6-OHDA model		AAV-aSyn model	
	Before injection (n=6)	After injection (n=6)	Stuffer vectors (control) (n=6)	aSyn coding vectors (n=5)
GABA	1.07 ± 0.03	1.20 ± 0.05**	1.03 ± 0.08	1.12 ± 0.01**
NAA	0.99 ± 0.02	0.93 ± 0.02*	0.97 ± 0.03	0.99 ± 0.02
Glu	1.01 ± 0.02	0.94 ± 0.01**	0.98 ± 0.02	1.00 ± 0.02
Lac†	1.25 ± 0.13	1.12 ± 0.12	1.01 ± 0.08	1.26 ± 0.12

*Table 1: Metabolite concentration ratios between the right and left hemisphere for each group. Values correspond to the mean of each animal group and the corresponding standard error of the mean. Their significance is indicated * (p<0.05) and ** (p<0.01). †Although no significant changes in Lactate (Lac) were noticed, we have included this metabolite in our analysis as it is often reported to be affected in conditions relevant to PD [3, 5].*

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