LESIONS OF VENTRAL TEGMENTAL AREA IN THE MOUSE AND CONSEQUENCES ON GLUTAMATE, GABA AND GLUTAMINE LEVELS ASSESSED USING PROTON 1H MRS.

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Purpose/Objective

The loss of dopaminergic (DA) neurons in the substancia nigra pars compacta induced changes in the neurochemistry of the dorsal part of the striatum at the origin of classical motor symptoms of Parkinson's disease (PD). An increase of glutamate (Glu), glutamine (Gln) and GABA levels is shown in the striatum of the mouse intoxicated with MPTP, animal model of PD [1]. DA neurons from the ventral tegmental area (VTA) which project to the nucleus accumbens (NAc) and which are involved in the mesocorticolimbic pathway also degenerate in PD. This DA denervation could be involved in the appearance of non-motor disorders (psychiatric and behavioral signs) described in some parkinsonian patients. Does this loss of DA neurons induce changes in the neurotransmitters levels in the NAc?

The aim of this study is to validate the in vivo quantification of cerebral metabolites in the NAc of mice with different DA denervation degrees using proton Nuclear Magnetic Resonance Spectroscopy (^{1}H MRS).

Methods

The study is performed on 10 control mice, 10 MPTP-intoxicated mice (25mg/kg, i.p. once daily during 5 days) and 10 6-hydroxydopamine (6-OHDA) lesioned mice (1 unilateral stereotaxic injection of 6-OHDA in the VTA, 4.5µg/1.5µL). MRS acquisitions are performed at 9.4T. Mice are anesthetized; the head was centered in birdcage coil (Ø 20mm). Spectra are acquired in a 1.56µL voxel (1.16^3 mm^3) centered in the NAc using PRESS sequence (TE=8.8ms; TR=4s; 1024 scans). A spectrum is acquired in the same conditions using the metabolite-nulling technique [2] to assess macromolecules contribution. After subtraction of the macromolecule spectrum, NMR spectra are fitted by jMRUI (QUEST) [3]. Metabolite levels are expressed as function of internal water content obtained from the unsuppressed water signal. After *in vivo* MRS exams, mice are sacrificed, their brains are included in paraffin and coronal sections 7µm thick are prepared from the VTA to quantify DA lesion by tyrosine hydroxylase (TH) immunolabeling.

Results

Fig. 1 shows a spectrum after subtraction of macromolecules acquired *in vivo* from the NAc of a control mouse. Results of the quantification are presented in the Fig. 2. Glu, Gln and GABA levels are significantly higher in the NAc of 6-OHDA-lesioned mice than in controls. MPTP intoxication induces no change in the NAc metabolites. The number of TH-positive neurons in the VTA decreases of 43% after MPTP intoxication ($p<0.05$) and 60% after 6-OHDA lesion ($p<0.001$). There is a significant correlation between the number of TH-positive neurons in the VTA and the levels of Glu, Gln and GABA measured in the NAc (Fig. 3). Higher the metabolite concentrations lower the number of neurons.

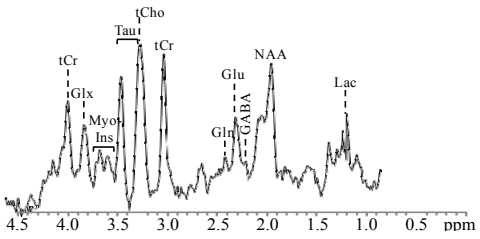

Fig. 1. *In vivo* spectrum acquired in the NAc of a control mouse.

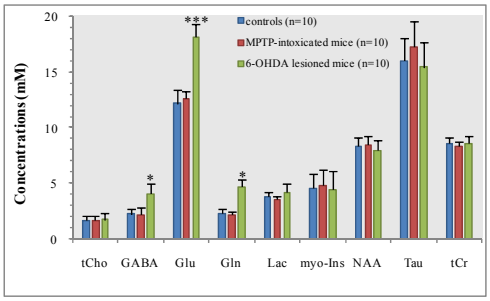

Fig. 2. Metabolite Concentrations . *p<0.05; ***p<0.001 vs controls

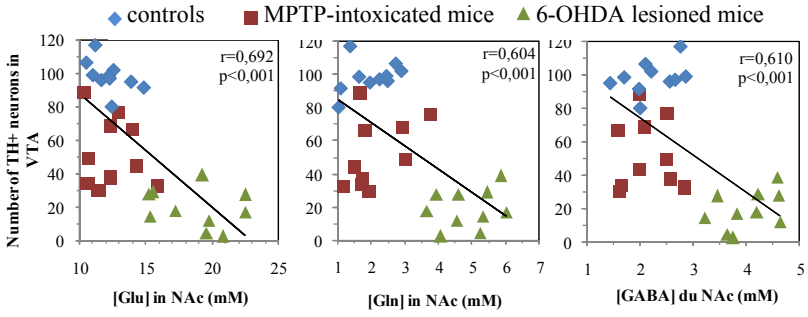

Fig. 3. Correlations between number of TH+ neurons in VTA and levels of Glu, Gln and GABA in NAc.

Discussion

In conclusion, we have validate the method to detect and quantify metabolite in a very small cerebral structure, the NAc of the mouse and we show that an important loss of DA neurons in the VTA is necessary to induce changes in the biochemistry of the NAc detectable by ^{1}H MRS. We will use this method to compare neurochemistry of NAc in animal model of PD with non-motor disorders and in animal model of PD without non-motor disorders.

References

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