# MR determination of neurochemical profile and TCA cycle flux reveals concomitant alterations of GABA/glutamine metabolism and oxidative pathway in a primate model of Huntington's disease

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## Introduction

Development of MR spectroscopy for clinical research depends to a large extent on the ability to obtain quantitative data from MR spectra. Two quantitative approaches have emerged for brain exploration over the last years: (i) the determination of neurochemical profile based on short echo time $^{1}$H spectroscopy and (ii) the measurement of metabolic fluxes such as the TCA cycle flux ($V_{TCA}$) based on $^{13}$C-isotopes. Although these approaches have been used to study brain disease [1,2,3,4,5], the question as to whether they are relevant for assessing long term neurodegenerative diseases is still open. In this context our primary purpose has been to evaluate both approaches over a 7-month longitudinal study of neurodegenerative process in primates. Chronic inhibition of succinate dehydrogenase (SDH) by the mitochondrial toxin 3-nitropropionic acid (3NP) was used as a primate model of Huntington's disease (HD) [6]. Our objectives were to determine the sensitivity of both methodological approaches to 3NP induced mitochondrial impairment and to test the hypothesis of a specific alteration of GABA metabolism under 3NP treatment.

## Materials and Methods

***Animals and 3NP treatment.*** Three macaque monkeys (*macaca fascicularis*, 7–8 kg) were studied before and during 3NP treatment (Sigma Aldrich Co., St Louis, MO, USA) for 30 weeks. The neurotoxic treatment consisted of two daily i.m. injections with an initial dose of 10 mg/kg/day (half dose at 9:00 am and 4:00 pm) progressively increased at weekly intervals to 30 mg/kg/day as previously described [7]. Then 3NP treatment was started and MRS measurements were performed at ~2 weeks intervals. Experiments were performed under propofol anaesthesia (i.v. infusion, 200 µg/kg/min), the animal being ventilated.

***Data acquisition.*** NMR Experiments were performed on a 3 Tesla whole-body system (Bruker, Ettlingen, Germany) equipped with a surface home-made $^{1}$H surface coil. T1 and T2 images were acquired for proper positioning of the 30×10×13mm$^3$ voxel in the striatum. After shimming down to 7 Hz, a baseline $^{1}$H PRESS spectrum was acquired (TE/TR = 8/2500ms, 432 scans) for the determination of neurochemical profile (11 experiments before intoxication, 29 during intoxication). For $V_{TCA}$ measurement, (6 exp. before intoxication, 18 during intoxication), $^{1}$H PRESS spectra were collected during an i.v. infusion of [U-$^{13}C_6$]glucose (3-min bolus, 120-min continuous infusion). Blood samples were collected for glucose $^{13}$C enrichment measurement.

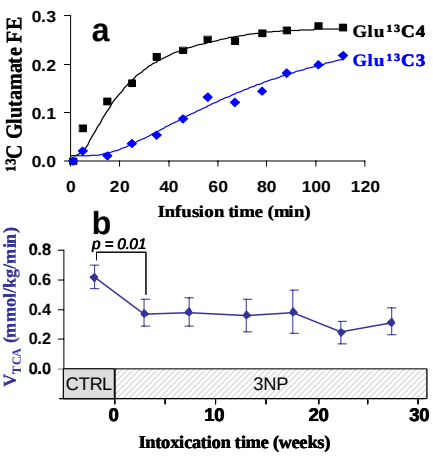


*Fig. 2. **a.** Glutamate $^{13}$C4 and $^{13}$C3 time-courses measured in one experiment and best fit. **b.** $V_{TCA}$ vs. 3NP intoxication time. The control value is the average from 6 measurements (2 per monkey), whereas each 3NP value is the average from 3 measurements (1 per monkey).*

***Neurochemical profile.*** The baseline PRESS spectrum was analyzed using LCModel, leading to the quantitation of the following metabolites: Asp, tCho, tCr, GABA, Glu, Gln, GSH, Ins, Lac, tNAA, Tau (Boumezbeur, this symposium).

***TCA cycle flux.*** $^{13}$C incorporation into brain glutamate C4 and C3 was detected on the $^{1}$H PRESS spectra as described elsewhere [8]. Time-courses of glutamate C4 and C3 fractional enrichment were fitted by a single compartment metabolic model describing $^{13}$C incorporation from glucose to brain glutamate [9], leading to $V_{TCA}$.

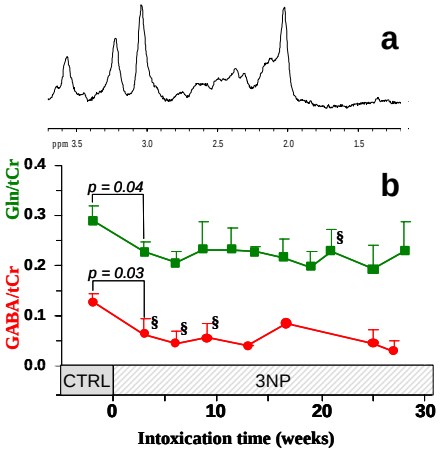


*Fig. 1. **a.** Example of $^{1}$H PRESS spectrum. **b.** Time-courses of GABA/tCr and Gln/tCr. Control values are averaged from 11 measurements. 3NP values are averaged from 3 measurements (1 per monkey) unless specified (§: av. from 2 monkeys).*

## Results

An example of baseline $^{1}$H PRESS spectrum of the monkey striatum is shown in Fig. 1a. Among the 11 metabolites quantified by LCModel, 9 metabolites exhibited non significant changes following 3NP intoxication. Comparison of tCr before and after 3 weeks of intoxication showed a 10% decrease, which could result either from increased water content (quantitation was based on voxel water signal) or from decreased intracellular concentration. Asp/tCr, GSH/tCr, Tau/tCr, tNAA/tCr, tCho/tCr, Ins/tCr, Lac/tCr and Glu/tCr presented non significant changes from control to week 3 (10 to 13% decrease, $p>0.09$, non-parametric Wilcoxon rank sum test). In contrast, GABA/tCr and Gln/tCr ratio exhibited strong and significant decreases (51% and 22% respectively, $p=0.03$ and $0.04$). Fig 2b presents metabolite times-courses measured over the protocol.

Glutamate $^{13}$C4 and $^{13}$C3 time-courses measured in one monkey during glucose infusion are displayed in Fig. 2a, with the best fits by the metabolic model. $V_{TCA}$ values over the 30-week intoxication protocol are displayed in Fig. 2b, exhibiting an immediate and highly significant 40% decrease upon intoxication ($p=0.01$, week 3 *vs.* pre-intoxication). The decrease was maintained all along the 3NP protocol (mean 44%, $p<0.00001$).

## Discussion

Previous baboon studies have reported significant NAA and Lac changes upon 3NP intoxication, associated with striatal lesions detected on T2-weighted MRI [10,11]. The lack of significant change in NAA and Lac in our study reflects the limited degenerative effect of the 3NP doses administered to our macaque. This explanation is supported by our observation of limited striatal lesions on T2 images: 1 monkey exhibited no lesion at all, whereas the 2 other monkeys exhibited delayed lesions (week 13 and week 24) which size did not exceed 1% and 5% of the spectroscopic voxel. Lac concentration was found steady around 0.5 mM in the 42 experiments conducted before and during the 3NP protocol, except for one single experiment characterized by the apparition of a lesion in the putamen ([Lac]=2 mM, week 24). In order to properly interpret changes in GABA and Gln, it must be reminded that striatum is mainly composed of GABAergic neurons, which means that neurotransmission relies on the GABA/Gln cycling. Therefore the selective decrease in GABA and Gln might reflect neurotransmission impairment.

The TCA cycle flux measured in the same striatal voxel demonstrated an immediate alteration of oxidative metabolism, in agreement with the decrease in glucose uptake measured by [$^{18}$F]-FDG PET (mean CMRglc decrease 46%, $p<0.01$, data not shown). These identical changes together with the lack of lactate increase argue against energy decoupling between oxidative and glycolytic pathways in our experimental conditions.

In conclusion combination of short echo time spectroscopy with $V_{TCA}$ measurement provides unique information for proper characterization of neurodegenerative processes. Impairment of mitochondrial function by 3NP leads to a selective alteration of GABA and Gln in the striatum, concomitant with a decrease in oxidative ($V_{TCA}$) and non-oxidative (CMRglc) metabolism. This observation might reflect an alteration of GABAergic neurotransmission, arguing in favor of a coupling with oxidative metabolism [12]. The extent to which GABAergic neurotransmission is altered in Huntington's patients remains to be explored.

**References:** 1. Pfeuffer et al., J. Magn Reson., 141, 104, 1999 2. Tkac et al., J. Neurosci. Res., 66, 891, 2001 3. Henry et al., J. Neurochem., 82, 857, 2002 4. Petroff et al., Epilepsia, 43, 703, 2002 5. Ross et al., NMR Biomed., 16, 358, 2003 6. Brouillet et al., Prog. Neurobiol., 59, 427, 1999 7. Mittoux et al., Hum. Gene Ther., 11, 1177, 2000 8. Boumezbeur et al., Magn. Reson. Med., 52, 33, 2004 9. Mason et al., J. Cereb. Blood Flow Metab., 12, 434, 1992 10. Dautry et al., Neurobiol. Dis., 6, 259, 1999 11. Dautry et al., J. Cereb. Blood Flow Metab., 20, 789, 2000 12. Shulman et al., Trends Neurosci., 27, 489, 2004