Glutamate CEST MRI in MPTP Mouse Model of Parkinson's Disease

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Target Audience: People interested in Molecular Neuroimaging, CEST MRI, and brain disorders

Introduction: Parkinson's disease (PD) is second most common neurodegenerative disorder affecting about 1-3% in people over 60 years of age. Sub-acute administration of neurotoxin 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine (MPTP) in mice is widely used to study mechanisms involved in the progression of PD[1]. It has been reported that selective dopaminergic cell death caused by MPTP leads to altered metabolite homeostasis in certain regions of the brain[2]. Chemical exchange saturation transfer (CEST) MRI allows indirect detection of metabolite concentration based on their exchange-related properties with bulk water[3]. Glutamate (Glu), the most abundant neurotransmitter in the brain and alterations in Glu are reported to occur in various neurodegenerative disorders[4]. Recently, GluCEST MRI has been applied in animals as well as humans to map small changes in regional cerebral Glu homeostasis[5,6,7]. The objective of the current study is to map the changes in regional Glu levels in the brain of mice treated with MPTP using GluCEST MRI and determine the correlation with (i) motor function loss measured from neurobehavioral tests and (ii) ^{1}H MRS derived [Glu] changes. These findings suggest a role for GluCEST as a biomarker of PD and for monitoring the efficacy of therapeutic treatment.

Methods: Two groups of male C57BL6 mice (Control (n=11) and MPTP treated (n=13)) were studied. MPTP treated mice received MPTP (25 mg/kg, i.p., Sigma Aldrich) for 7 days and Control mice received normal saline during the same period. Loss of motor function due to MPTP administration was assessed by performing the forepaw grip strength test and the four limb endurance test. For MR studies, mice were anesthetized with Isoflurane/O_2 (1-2%, 1 L/hr) and positioned in a 9.4T Horizontal bore (Agilent Technologies Inc., Santa Clara, CA) spectrometer. ^{1}H MRS was performed in striatum using a 20 mm volume coil and PRESS pulse sequence (voxel size 2.25×2.25×2.25 mm^3, TE/TR 28/3000 ms, 512 averages). Metabolite levels were quantified with LCModel using unsuppressed water signal as reference. GluCEST imaging of striatum and thalamus was performed using a custom-programmed RF spoiled gradient echo readout pulse sequence in 2mm slices with a frequency selective continuous wave saturation preparation pulse. CEST images were collected using a 1 second saturation pulse at peak B_1 of 250 Hz for the frequencies ±2.5-3.5 ppm from water resonance with step size of 0.25 ppm. The B_0 inhomogeneity corrected images at 3 ppm (M_{+3ppm}) and -3 ppm (M_{-3ppm}) were used for computing the percent GluCEST contrast, which is equal to $100\times[(M_{-3ppm} - M_{+3ppm})/M_{-3ppm}]$. Regions of interest were manually segmented from T_2-weighted images. Student's t-test was used to determine the significance of difference between the two groups.

Results and Discussion: MPTP treatment led to a significant loss in motor function measured by the forepaw grip strength test (Fig 1A) and the four limb hanging test (Fig 1B). Analysis of ^{1}H MR spectra using LCModel showed a significant increase (~17%) in the level of striatal Glu (Control 12.6±1.7 µmol/g MPTP 14.8±2.6 µmol/g; p=0.0013) following MPTP treatment (Fig 2A,B). There was no alteration in the levels of striatal NAA, Cre, taurine and choline in the MPTP group (Fig 2B). GluCEST maps clearly show higher GluCEST contrast in the striatum of MPTP treated mice as compared to control group (Control 23.3±1.5 %, MPTP 26.3±1.1 %; p=0.00005). Interestingly, GluCEST contrast was found to be mostly unaltered in thalamus of MPTP treated mice (Control 25.0±1.8 %, MPTP 26.2±2.5 %; p=0.34). In agreement with the ^{1}H MRS results, the increase in GluCEST contrast was found to be ~13 % over the control value. Overall, MPTP treatment resulted in ~1.4% elevation of striatal GluCEST per mM Glu as measured from ^{1}H MRS. GluCEST contrast elevation was also associated with significantly decreased motor function of the MPTP treated mice. These findings are consistent with previous studies[2,6]. GluCEST MRI demonstrates elevated striatal Glu with high spatial resolution and can potentially provide a biomarker for the detection of PD neuropathology in humans. Additionally, GluCEST MRI may be used to assess the efficacy of novel therapeutic interventions and treatment modalities for PD and related neurodegenerative disorders in humans.

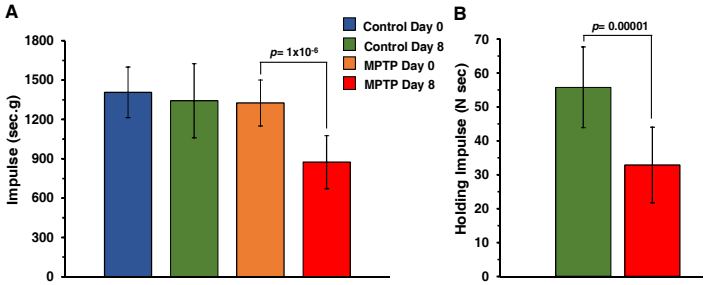

Fig. 1. **A.** Fore paw grip strength test and **B.** Four limb hanging test showing significant reduction in the motor function following administration of MPTP

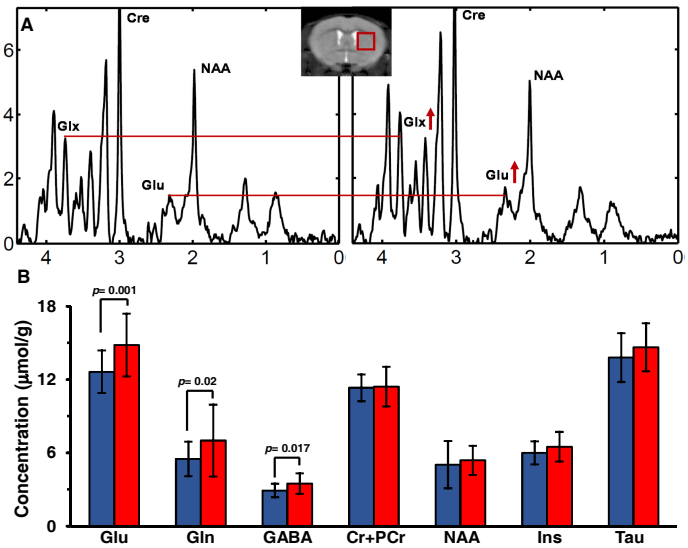

Fig. 2. **A.** ^{1}H MR spectra from striatum of control (left) and MPTP treated (right) mice showing elevated level of Glu. **B.** Concentration (µmol/g) of metabolites in striatum using LCModel depicting higher Glu levels

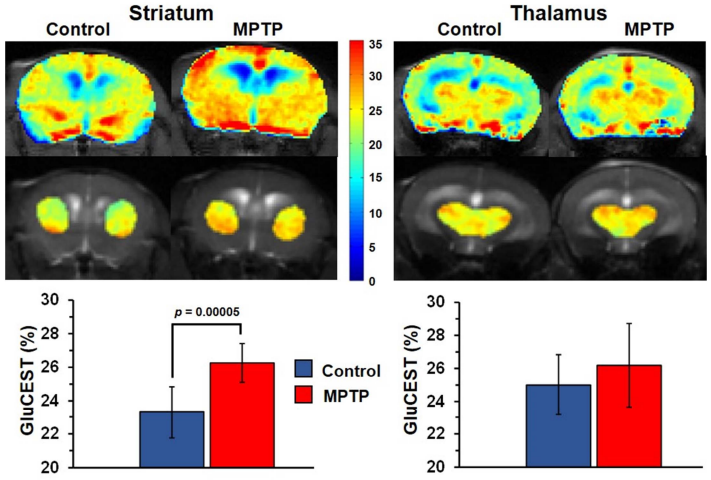

Fig. 3. GluCEST maps from axial slices in striatum and thalamus regions of the brain. GluCEST contrast was found to be elevated in the striatum (Left) of mice treated with MPTP, while it was unaltered in the thalamus (Right)

References: 1. Araki T *et al* (2001) *Eur J Pharm Sci* **12**:231-38; 2. Bagga P *et al* (2013) *J Neurochem* **127**:365-77; 3. Kogan F *et al* (2013) *Curr Radiol Rep* **1**:102-14; 4. Lau and Tymianski (2010) *Pflugers Arch* **460**:525-42; 5. Cai K *et al* (2012) *Nat Med* **18**:302-6; 6. Haris M *et al* (2012) *NMR Biomed* **26**:386-91; 7. Crescennzi R *et al* (2014) *Neuroimage* **101**:185-92

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