Investigation on Neuroprotective Role of Caffeine against MPTP Induced Neurotoxicity in Mice using $^{13}$C NMR Spectroscopy
Puneet Bagga$^1$, Suresh Kumar M$^1$, and Anant Bahadur Patel$^1$
$^1$NMR Microimaging and Spectroscopy, Centre for Cellular and Molecular Biology, Hyderabad, Andhra Pradesh, India

Introduction: Parkinson’s disease (PD) is a debilitating disorder of the human brain affecting 0.3% of the entire population and about 1-3% in people over 60 years of age. Neurotoxin MPTP has been used in rodent as well as non-human primate models for studying mechanisms involved in PD$^1$. Caffeine uptake is negatively correlated with occurrence of PD in humans$^2$. The objective of the current study was to evaluate the neuroprotective effect of caffeine in mouse model of MPTP neurotoxicity on regional glutamatergic and GABAergic function by using $^1$H-$^{13}$C-NMR spectroscopy together with infusion of [1,6-$^{13}$C$_2$]glucose.

Materials and Methods: All experiments were performed under protocols approved by the Institute Animal Ethics Committee. Three groups of four month old male C57BL6 mice were studied: Group A: Control (n=11); Group B: MPTP treated (n=13); Group C: Caffeine + MPTP treated (n=5). Group B mice were treated with MPTP (25 mg/kg, i.p.) for 8 days while Group C mice received caffeine (30 mg/kg, i.p.) 30 min prior to administration of MPTP and control mice (Group A) received normal saline only. For metabolic studies, overnight fasted mice were anesthetized with urethane and infused with [1,6-$^{13}$C$_2$]glucose intravenously for 10 min$^3$. At the end of the infusion, brain was frozen in situ in liquid N$_2$ and metabolites were extracted from frozen brain regions$^4$. $^1$H-$^{13}$C-NMR spectra were acquired from tissue extracts for the measurement of concentration and $^{13}$C enrichment of amino acids$^5$.

Results and Discussion: Level of GABA and glutamine was found to be increased significantly (p<0.05) in striatum after chronic MPTP treatment which was not significantly different in mice treated with caffeine before MPTP administration. Cerebral metabolic study revealed that the $^{13}$C labeling of GluC$_4$, GABA$_{C2}$ and GlnC$_4$ from [1,6-$^{13}$C$_2$]glucose was decreased significantly (p<0.01) in cortex, thalamus-hypothalamus, striatum and olfactory bulb indicating an impairment in glutamatergic and GABAergic TCA cycle and neurotransmission after treatment of MPTP. Pretreatment with caffeine led to a recovery of $^{13}$C labeling of amino acids indicating protection of neural function (glucose oxidation and neurotransmission) in cortex and olfactory bulb, while a partial protection was observed in striatum and thalamus-hypothalamus. Hence, it could be concluded that caffeine can partially act as a neuroprotectant against the MPTP induced neurotoxicity.

Fig. 3. Concentration of $^{13}$C labelled amino acids from [1,6-$^{13}$C$_2$]glucose at 10 min in A. Cortex and B. Thalamus-hypothalamus. Values of GABA$_{C2}$ & GlnC$_4$ are presented as twice of the observed.


Acknowledgements: This study was supported by funding from Department of Science and Technology, India.