^{1}H-[^{13}C]-NMR Studies of Cerebral Metabolism in Chronic Unpredictable Mild Stress Model of Depression

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INTRODUCTION: Depression and related mood disorders are usually associated with severe and persistent symptoms leading to high morbidity rates and increased mortality[1]. The etiology of major depressive disorder is complex and includes perturbations in brain circuits, alterations in various hormones, neurogenesis, angiogenic factors, neurotrophic factors, and monoaminergic transmission[2]. However, cerebral metabolism under depression is still not clear. Prefrontal Cortex (PFC), a brain area critical for complex cognitive functions such as decision making, is also known to be involved in reward response as well as regulation of emotion, and plays an important role in depression[3]. In this study, we have evaluated neuronal and astroglial metabolism in chronic unpredictable mild stress (CUMS) model of depression using ^{1}H-[^{13}C]-NMR spectroscopy together with infusion of [1,6-^{13}C$_2$]glucose and [2-^{13}C]acetate.

MATERIALS AND METHODS: All animal experiments were performed under approved protocols by the Institutional Animal Ethics Committee. Two groups of male C57BL6 mice (2 month old) were studied: Group (i) Control (n=12), Group (ii) CUMS (n=13). Mice in Group (ii) were subjected to different stresses such as cold swim, wet bedding, tilt cage, tail pinch, tail suspension, density crowding, etc, twice a day for 21 days[4]. The functioning of brain reward circuit in mice was assessed by the sucrose preference test[5] and forced swim test (FST). For metabolic measurements, overnight fasted mice were anesthetized with urethane (1.5 g/kg, i.p). [1,6-^{13}C$_2$]Glucose was administered via tail vein into mice (n=6 each group) for 10 minutes using a bolus-variable rate infusion protocol[6]. In addition, both groups of mice were infused with [2-^{13}C]acetate for 15 min to evaluate the effects of CUMS on astroglial metabolism[7]. At the end of infusion, blood was collected and mice head were frozen in liquid nitrogen. PFC was dissected and metabolites were extracted from frozen tissue[8]. The concentration (Fig. 1A) and percent ^{13}C enrichment (Fig. 1B) of metabolites were determined from the ^{1}H-[^{13}C]-NMR spectrum of the PFC extract recorded at 600 Hz Bruker AVANCE spectrometer[9].

Fig. 1 (A) ^{1}H-[^{12}C+^{13}C]-NMR and ^{1}H-[^{13}C]-NMR spectra from PFC extract of CUMS mice showing ^{13}C from (B) [1,6-^{13}C$_2$]gluocse and (C) [2-^{13}C]acetate

RESULTS AND DISCUSSIONS: Animal subjected to three weeks of CUMS exhibit significant reduction in the sucrose preference test (Control 89.6±1.7%; CUMS 50.5±22.1%; p<0.0001). Furthermore, FST indicated significant (p=0.001) increased immobility in CUMS (32.2±11.6%) as compared with control mice (16.0±8.2%) (Fig. 2A). Both behavioral assays clearly showed depression-like phenotype in mice subjected to CUMS. The level of PFC metabolites was unperturbed with 3 weeks CUMS. However, mice subjected to CUMS exhibited significant reduction in the labeling of Glu$_{C4}$, GABA$_{C2}$ and Gln$_{C4}$ from [1,6-^{13}C$_2$]glucose, suggesting decreased PFC metabolism in CUMS mice. Metabolic rate of glucose oxidation derived from the ^{13}C labeling of neurotransmitters, indicated significant reduction in glucose oxidation by glutamatergic (Control 0.28±0.04 µmol/g/min; CUMS 0.20±0.04 µmol/g/min; p=0.005) and GABAergic neurons (Control 0.06±0.004 µmol/g/min; CUMS 0.04±0.01 µmol/g/min; p=0.02) (Fig. 2B). The ^{13}C labeling of Glu$_{C4}$, GABA$_{C2}$ and Gln$_{C4}$ from [2-^{13}C]acetate (Fig. 1C), an astroglial specific substrate, was also significantly reduced in CUMS mice (Fig. 2B). Thus, the rate of acetate utilization was reduced in the PFC of CUMS mice (Control 0.17±0.003 µmol/g/min; CUMS 0.12±0.01 µmol/g/min; p<0.0001). The reduction in the neuronal activity has been reported in the social defeat model of depression[10]. The reduced glucose oxidation by glutamatergic and GABAergic neurons together with reduced acetate utilization suggest that both neuronal and astroglial activities are reduced in CUMS model of depression.
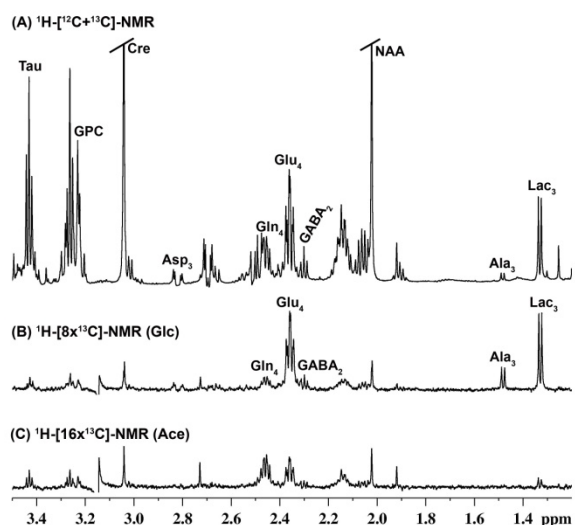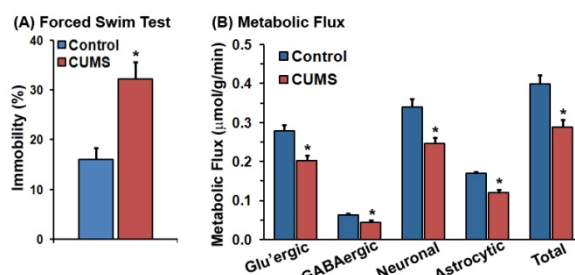

Fig. 2 (A) Percent immobility, (B) Metabolic Flux in control and CUMS mice

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