Evaluation of Efficacy of Ketamine for the Treatment of Depression like Phenotype in Mouse Model: A ^{1}H-[^{13}C]-NMR study

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Introduction: Major depressive disorder (MDD) is a complex debilitating neuropsychiatric disorder and includes perturbations in monoaminergic neurotransmission, neurogenesis, hormone levels[1]. Glutamate (Glu) and γ-amino butyric acid (GABA) are major excitatory and inhibitory neurotransmitters in cortical region. A very recent study has indicated reduction in glutamatergic and GABAergic activity in the social defeat model of depression[2]. The major problem with current antidepressants is long time lag (weeks to months) for remission, which may lead to increased morbidity and suicidal behavior. Clinical studies have consistently demonstrated that a single sub-anesthetic dose of ketamine, an ionotropic glutamatergic N-methyl-D-aspartate receptor antagonist, produces fast antidepressant response in patients suffering from major depressive disorder, although the underlying mechanism is unclear[3]. The current study investigates the response of ketamine in behavioral phenotype, and cerebral energy metabolism in the social defeat model of depression.

Materials and Method: All animal experiments were carried out with approved protocols from the Institutional Animal Ethics Committee. Two groups of male C57BL6 mice (2 month old) were used for the study: Group (i) control mice, Group (ii) Social defeat. Mice in Group (ii) were subjected to social defeat protocol for 10 days[4]. After 10 days of social defeat, each group was further divided into two subgroups and treated with either ketamine (10 mg/kg, i.p.) or normal saline. Mice were subjected to sucrose preference and social interaction test before and after ketamine administration for the assessment of depression phenotype[5]. For the metabolic measurement; mice were fasted for 6 hours and anesthetized with urethane. [1,6-^{13}C$_2$]Glucose was administered using a bolus variable infusion protocol for 10 minutes[6]. At the end of infusion period, mice head were frozen into *liq.* nitrogen and metabolites were extracted from the frozen prefrontal cortex (PFC)[7]. The concentration and percentage ^{13}C enrichment of amino acids were measured in ^{1}H-[^{13}C]-NMR spectrum (Fig. 1A) of PFC extract acquired at 600 MHz spectrometer[8]. Glucose oxidation by glutamatergic and GABAergic neurons were calculated from the initial rate of labeling from [1,6-^{13}C$_2$]glucose[9].

Results and discussion: Mice subjected to social defeat were showing significantly reduced (p<0.01) sucrose preference (Control 86.9±1.6%; SD 63.6±3.1%) and social interaction (Control 152±13%; SD 68±8%). Intervention with ketamine improved the sucrose preference (SD+NS 63.6±3.1%; SD+KT 85.2±2.7) as well as social interaction (SD+NS 68±8%; SD+KT 157±37%) in SD mice (Fig. 1). The concentration of PFC metabolites obtained from ^{1}H-[^{12}C+^{13}C]-NMR spectrum (Fig. 2A) did not show significant difference among various groups. The incorporation of ^{13}C label into amino acids in SD mice was found to be significantly reduced (p<0.01) than control suggesting metabolism in PFC is impaired in depressed mice. Acute treatment of ketamine normalizes the labeling to the control value. The cerebral metabolic rate of glucose oxidation, a measure of cellular activity, was significantly reduced (p<0.01) for glutamatergic and GABAergic neurons in SD mice (Fig. 3). The reduced glucose oxidation associated with glutamatergic and GABAergic neurons could recover to control level following acute treatment of ketamine in SD mice. Together, these data indicate that sub-anesthetic exposure of ketamine improved the reward circuitry, and excitatory and inhibitory neurotransmitter activity in social defeat model of depression.
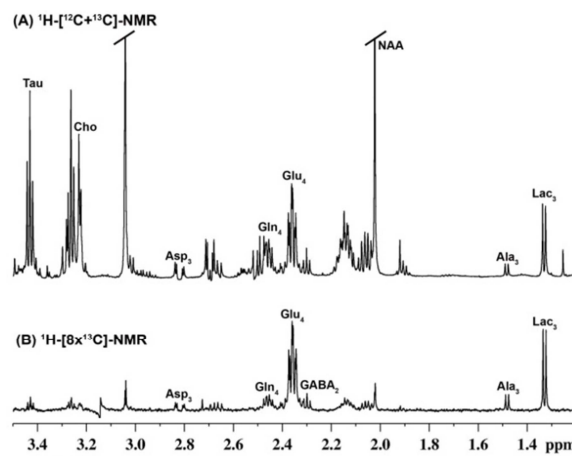

Fig. 1 Behavioral phenotype during various interventions

Fig. 2 ^{1}H-[^{13}C]-NMR spectra from PFC extract showing (A) concentration, (B) ^{13}C labeling from [1,6-^{13}C$_2$]glucose in ketmine treated mouse
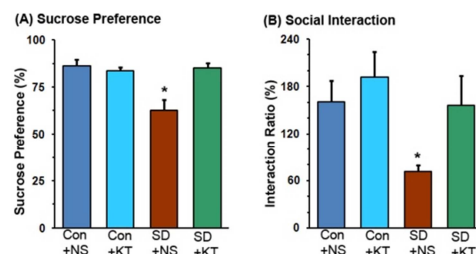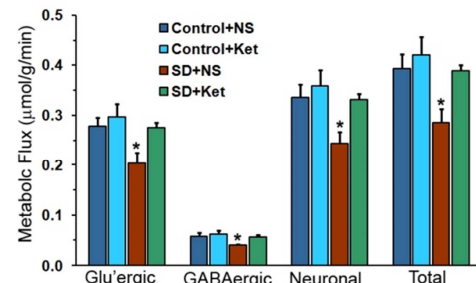

Fig. 3 Metabolic rate under different interventions

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