

# In vivo evidence for ketamine-induced neurochemical changes in rat prefrontal cortex: an animal model of schizophrenia

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## INTRODUCTION

Although the etiology and pathophysiology of schizophrenia have not yet been elucidated, consistent clinical observations associated with a pharmacological challenge with N-methyl-D-aspartate (NMDA) antagonists provide direction for formulating new pharmacological models of the illness. Subanesthetic doses of ketamine, a noncompetitive NMDA receptor antagonist, impair prefrontal cortex (PFC) function in the rat and produce symptoms in humans similar to those observed in schizophrenia [1]. This has led to the suggestion that schizophrenia may involve hypofunction of NMDA receptors or glutamate neurotransmission. There is currently no information available regarding the effects of chronic subanesthetic dose of ketamine on neurochemical responses in rat brain *in vivo*. In the present study, we used *in vivo* and *in vitro* <sup>1</sup>H-NMR spectroscopy to examine the brain metabolism of rat treated with chronic subanesthetic dose of ketamine and hypothesized that the chronic injection of ketamine would result in changes in MR-observable metabolite involved in neurotransmission (glutamate, glutamine).

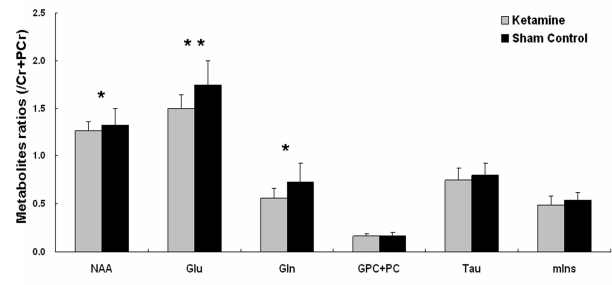
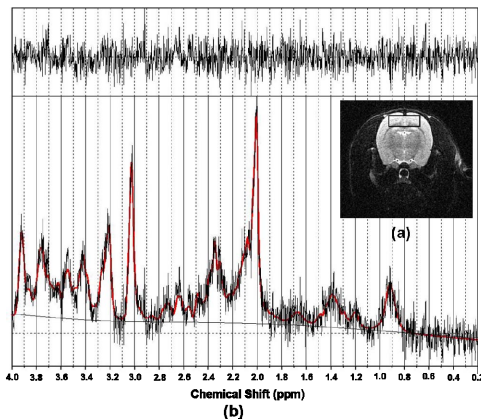
## MATERIALS AND METHODS

**Animal treatment** Male Sprague-Dawley rats (160-180g, N=20) were divided into two groups [ketamine (N=10), saline (N=10)]. The ketamine group received once daily subcutaneous injection of ketamine (30 mg/kg) for 6 days. The control group received only saline (1 ml/kg).

**In vivo <sup>1</sup>H-MRS acquisitions and quantification** *In vivo* MR experiments were conducted using a 4.7 T BIOSPEC scanner (Bruker Medical GmbH, Ettlingen, Germany). A single voxel localization sequence (PRESS, TR/TE = 4000/20 ms, NEX=512, scan time=30 min) was used to acquire spectra in a 30  $\mu$ l voxel positioned in PFC area of the rats anesthetized with isoflurane. The metabolites were quantified using the LCModel with basis set including 17 metabolites (Ala, Asp, Cr, GABA, Glc, Glu, Gln, GSH, GPC, PCho, mIns, Lac, NAA, NAAG, PCr, Scy and Tau). Metabolite spectra were quantified as ratios to Cr + PCr.

**Tissue extraction and in vitro NMR spectroscopy** After *in vivo* MRS acquisitions, the rats were sacrificed and the PFC of brain for control (N=5) and ketamine-treated rat (N=5) were quickly removed (< 5 min). *In vitro* MRS acquisitions were performed on methanol-chloroform-water extracts of PFC part of rat brains. The data were acquired with a standard one-pulse experiment (spectral width=8000 Hz, TR=5 sec and average of 128 scans) at 11.7 T (500 MHz for <sup>1</sup>H) using Varian Inova spectrometer. *In vitro* NMR spectra were fitted by AMARES algorithm within JMRUI software. The metabolite concentrations were calculated from a comparison of their signal amplitudes with the amplitude of the internal reference TSP.

**Fig. 1.** Coronal RARE image of the rat brain with the VOI (a) and representative *in vivo* <sup>1</sup>H-NMR spectrum measured from prefrontal cortex (PFC) of rat (b). The spectra were fitted by LCModel (red line) and residue signal is displayed on top of the plot.



**Fig. 2.** *In vivo* proton metabolite ratios of the PFC of rat treated ketamine or saline (sham control) quantified by LCModel. Significance level: \*P < 0.05, \*\*P < 0.005

## RESULTS

Fig. 1 shows a coronal MR image of the rat brain, on which the PFC area was identified for acquisition of spectroscopic data, and displays a representative *in vivo* proton spectra fitted by LCModel. The lower metabolic levels of NAA, Glu, Gln were observed in chronic ketamine-treated rat group as compared to control (Fig. 2). *In vitro* NMR spectrum of brain extracts of ketamine or saline-treated rats are shown in Fig. 3. Unlike to the *in vivo* data, the metabolic concentrations of NAA, Glu, Gln were not significantly different between the two groups (data are not shown). *In vivo* and *in vitro* data showed that no significant differences between the two groups were found for the other metabolites.

## DISCUSSION AND CONCLUSION

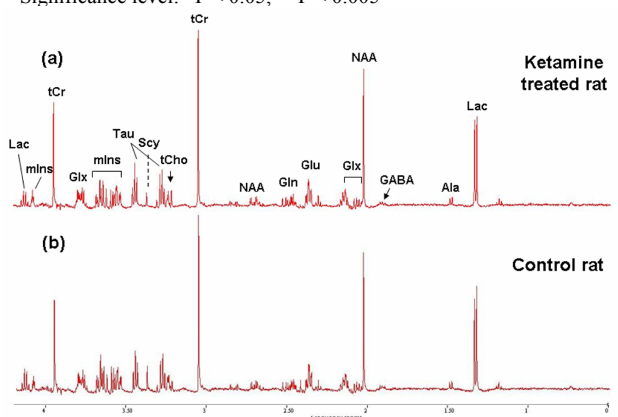
The present study demonstrated the effects of chronic injection of ketamine on neurochemical responses in rat PFC area using *in vivo* and *in vitro* NMR spectroscopy. *In vivo* data for Glu/Gln abnormalities in ketamine-treated rats may support the hypotheses of glutamate dysfunction for schizophrenia. In addition, lower metabolic level of NAA in rats treated with ketamine may indicate reduced neuronal viability, which is consistent with extensive clinical MRS research [2]. Therefore, our findings suggest that the neurochemical alterations induced by NMDA antagonists may provide the foundation for pathophysiological models of schizophrenia and experimental paradigms to explore mechanisms of antipsychotic drug actions.

## ACKNOWLEDGEMENT

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## REFERENCES

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**Fig. 3.** *In vitro* solution <sup>1</sup>H-NMR spectra from brain extracts of rat [(a) ketamine (b) saline treated rat]. The spectrum is acquired using CPMG pulse sequence (spectral width= 8000 Hz, TR= 5 sec, NEX=128) at 11.7 T. All metabolites were referenced to the 3-trimethylsilyl-propionic acid (TSP, 0.05 %).