# Sex-linked difference of metabolic concentrations in hippocampus in Sprague-Dawley rats: a high resolution *in vivo* proton MRS study at 7 Tesla

Su Xu[1,2], Andrew D Marshall[1,2], Nicholas Hilker[1,2], and Rao P Gullapalli[1,2]

[1]*Department of Diagnostic Radiology and Nuclear Medicine, University of Maryland School of Medicine, Baltimore, Maryland, United States,* [2]*Core for Translational Research in Imaging @ Maryland, Baltimore, Maryland, United States*

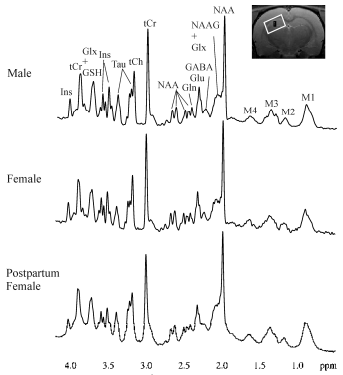


Fig 1. *In vivo* high resolution $^1$H spectra and corresponding voxel location depicted on the anatomic images of the left hippocampus from male, virgin female, and post-weaning female rats (2.5 x 4.0 x 3.0 mm$^3$). Each spectrum represents the sum of the total eight animals in the group.

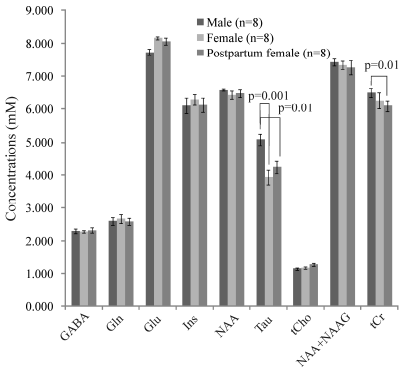


Fig 2. Comparison of the concentrations of the metabolites in hippocampus in male, virgin female and post-weaning female rats. Data are expressed as mean ± standard error.

## Introduction

Although high resolution localized $^1$H MRS has been widely used to study normal and pathological rat brain metabolism, very little has been reported on gender based metabolic differences among normal healthy rodents. Understanding these differences will allow accurate assessment of therapeutic regimens relying on metabolic markers obtained from MRS. The purpose of this study was to compare the metabolic concentrations obtained from normal age-matched Sprague-Dawley (SD) male, virgin female, and post-weaning SD rats in hippocampus, which is a major component of the brains of humans and other vertebrates and has been studied extensively in neuro- physiology and pathology.

## Materials and Methods

SD rats, male (n=8), and virgin female (n=8) that were 10-12 weeks old, and post-weaning female (n=8) 12-24 days postpartum were used in the study. *In vivo* $^1$H MRS experiments were performed on a Bruker BioSpec 7T horizontal bore MR scanner. A Bruker four-element $^1$H surface coil array was used as the receiver and a 72 mm linear-volume coil as the transmitter. Rats were anesthetized in an animal chamber using a gas mixture of $O_2$ (1 L/min) and isoflurane (3.5 - 4 %) and then later maintained at 1-1.5% isoflurane during scanning. A MR compatible small-animal monitoring and gating system was used to monitor the animal respiration rate and body temperature.

Proton density-weighted images were obtained using a 2D rapid acquisition with relaxation enhancement (RARE) sequence in the axial plane ($TR/TE_{eff}$ = 4500/28 msec, RARE factor = 4, field of view = 35 x 35 mm$^2$, slice thickness = 1 mm, in-plane resolution = 135 x 135 μm$^2$, number of averages = 1) and were used as anatomic reference for the MRS data acquisition. A customized short-TE PRESS pulse sequence (TR/TE = 2500/10 ms, number of average = 356)[1] was used for MRS data acquisition rom the left hippocampus (2.5 x 4.0 x 3.0 mm$^3$). LCModel package was used for quantification of the MRS data. The reliability of the major metabolites was estimated in the Cramér-Rao lower bounds (CRLB) from the LCModel analysis. The experimental protocol was approved by the Committee for the Welfare of Laboratory Animals of the University of Maryland. Unpaired two-tail T-test was used for statistic analysis.

## Results

Fig 1 demonstrates *in vivo* high resolution $^1$H spectra from the left hippocampus from male, virgin female, and post-weaning female rats. Each spectrum represents the sum of the total eight animals in the group. A number of metabolites were reliably detected (CLRB < 6%) including glutamate (Glu), myo-inositol (Ins), *N*-acetylaspartate (NAA), taurine (Tau), total Choline (tCho), NAA+ *N*-acetylaspartateglutamate (NAAG), and total Creatine (tCr). Some of the relatively lower concentration metabolites such as γ-aminobutyric acid (GABA) and glutamine (Gln), were also detected with a CRLB of about 12 %. Among the metabolites, Tau demonstrated significant sex differences (male vs. virgin female $p < 0.0003$; male vs. post-weaning female $p < 0.01$) (Fig 2). Tau concentration in virgin female rats (3.94 ± 0.13 mM) was 23 % lower than that in male rats (5.08 ± 0.18 mM). Although the post-weaning female rats (4.29 ± 0.18 mM) had a higher Tau concentration compare to virgin female rats, the concentration was still 16 % less than the male rats (Fig 2). In addition to Tau, tCr concentrations were lower in the post-weaning female rats, but not in the virgin rats, compare to the male rats. No statistically significant differences were found in GABA, Gln, Glu, Ins, tCho, NAA and NAA + NAAG concentrations among the three groups. There was no significant difference in any of the metabolites between virgin female, and post-weaning female groups.

## Discussions and Conclusions

In this study we demonstrated a sex-linked difference of metabolic Tau concentration in hippocampus in Sprague-Dawley rats. Both virgin and post-weaning female rats demonstrate significantly low Tau levels compared to the male rats. Tau is one of the most abundant amino acids (1-10 mM) in mammals. The role of Tau has been shown to be involved in many important physiological functions, e.g., as a trophic factor in the development of the central nervous system[2], maintaining the structural integrity of the membrane[3], regulating calcium binding and transport[4,5], as an osmolyte[6-8], as a neuromodulator[9], as a neurotransmitter[10], and as a neuroprotector against glutamate-induced excitotoxicity[11-13]. In hippocampus, Tau is most likely released from interneurons, inhibits firing of the main pyramidal neurons, and is a part of the hippocampal glutamatergic excitatory circuit[14]. Tau is a structural analog of the inhibitory neurotransmitters GABA, and activates GABAA receptors in several brain regions including hippocampus[15]. The osmoregulatory and neuroinhibitory actions of Tau may both thus affect neuronal development, differentiation and protection against hypoxic, oxidative and excitotoxic insults[13,16]. Indeed, Tau regulates neuronal activity, particularly in the immature brain[13, 17], and protects neural cells against the toxicity of excitatory amino acids in the hippocampus[18]. The neurophysiological basis for sex differences in Tau levels in hippocampus of SD rats is unclear. Studies have shown that neuroactive steroids such as estrogen, progesterone, testosterone, and dehydroepiandrosterone sulphate (DHEAS) regulate almost all kinds of classical synaptic transmission, including glutamatergic, GABAergic, cholinergic, noradrenergic, dopaminergic and serotonergic synaptic transmission, by altering the responsiveness of postsynaptic receptors or the presynaptic release of neurotransmitter[19]. In our current study, the post-weaning female rats showed a slightly elevated Tau level compare to the virgin female rats, but were still significantly below the levels seen in male rats. Because Tau plays a similar neuronal inhibitor role as GABA in the brain, it may also be regulated by the neuroactive steroids under different physiological conditions including during pregnancy. However, it seems that the big Tau gap between the male and female cannot be explained simply by the hormonal regulation. In addition, low tCr level in the post-weaning female rats compare to male rats may indicate energy depletion related to the weaning process.

In conclusion, the findings of our present study emphasize the importance of gender-matching in cross-sectional studies investigating differences in neurometabolic concentrations related to various neuro-developmental disorders.

## References

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