Neurobiological Assessment of Stress-Induced Sleep Disturbance in a Rat Model using In Vivo Proton Magnetic Resonance Spectroscopy at 9.4 T: Potential Relevance to Insomnia

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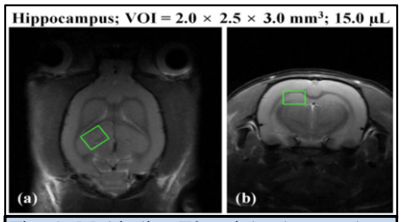

Fig. 1. Multi-slice T2-weighted coronal (a) and axial (b) MR images (TR/TE = 4000/33 ms) of the rat brain with the VOI centered in the right dorsal hippocampal region.

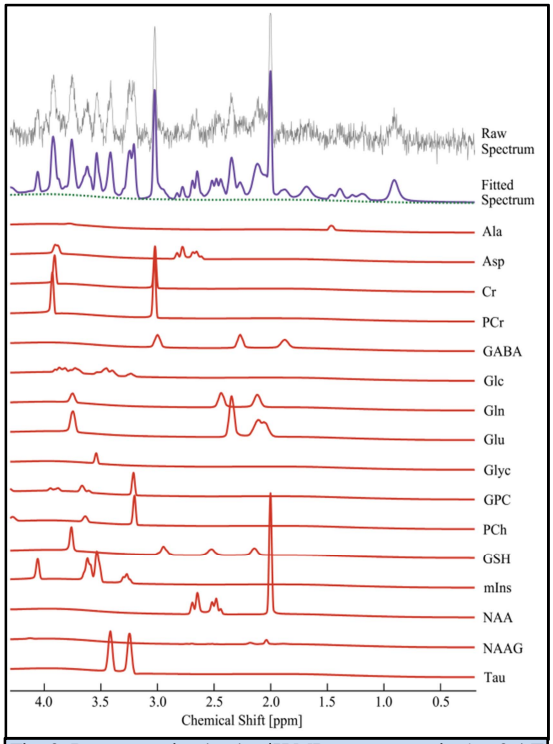

Fig. 2. Representative *in vivo* ^{1}H MR spectra acquired at 9.4 T from stress-induced sleep perturbed rats in the hippocampal region. Quantified spectra are represented by several colors, as follows: Fitted spectra (purple), raw spectrum (grey), baseline (dotted green), and metabolite signals (red).

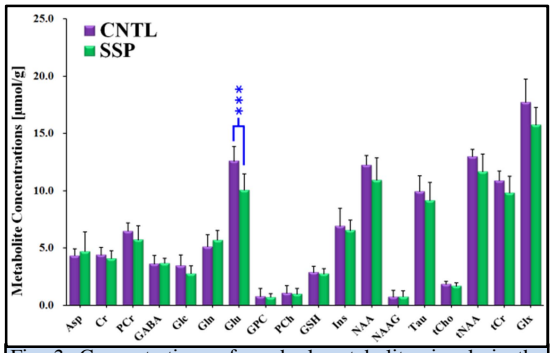

Fig. 3. Concentrations of cerebral metabolite signals in the dorsal hippocampal region of control (n = 7) and stress-induced sleep perturbed (n = 7) rats. Metabolite concentrations are expressed as micromole per gram (µmol/g). Independent t-tests were considered significant at *** $p < 0.005$. Vertical bars indicate the positive standard

Target audience: Neurologist, psychiatrist, medical doctors, and clinicians interested in MRS of the brain disorders.

Purpose: The hippocampus is an important brain region for regulating stress responses and is highly sensitive to the neurotoxic effects of cerebral metabolism changes.[1] Neuronal degeneration of the dorsal hippocampal region upon exposure to stress has been reported in animal assessments and in psychiatric disorders related to stress-induced events.[2] In particular, stress-related sleep disruptions (insomnia) might lead to persistent alterations in cerebral metabolism and abnormal neurochemical responses.[3] Therefore, this study aimed to quantitatively assess the neurobiological changes and responses, after stress-induced sleep disturbance in rats using *in vivo* proton magnetic resonance spectroscopy (^{1}H-MRS) at 9.4 T.

Methods: Male Sprague-Dawley rats (8-weeks-old, n = 14) were divided into two groups (sham control rats [CNTL]: n = 7; stress-induced sleep perturbed rats [SSP]: n = 7). SSP rats were placed in individual cages for a week without cleaning and then placed at 11:00 A.M. into a dirty cage previously occupied by another male rat for a week (cage exchange). All SSP rats were left undisturbed in the previously occupied cage (dirty cage) until the start of the ^{1}H-MRS spectra acquisition (5.5 h after the cage exchange). Seven CNTL rats were placed in clean cages at the same time (cage exchange at 11:00 A.M.) to synchronize the ultradian cycles between the two groups. The MR images and spectroscopy data were acquired using a 38-mm 4-channel quadrature phased array coil, and 72-mm volume coil. For volume of interest (VOI) localization and identification of anatomical regions, multi-slice T2-weighted MR images were acquired using a fast spin echo (FSE) pulse sequence (TR = 4000 ms, TE_{eff} = 33 ms, ESP = 10.98 ms, ETL = 32, average = 1, FOV = 30 × 30 mm, slice thickness = 1 mm, matrix size = 256 × 256, total scan time = 2 min 16 sec). The VOI (2.0 × 2.5 × 3.0 mm = 15.0 µL) position was targeted to the dorsal hippocampal region [Figure 1(a) and (b)]. Water suppressed *in vivo* ^{1}H-MRS spectra were acquired using a point-resolved spectroscopy (PRESS) pulse sequence with a variable power and optimized timing (VAPOR) method (TR = 5000 ms, $TE_1/TE_2/TE_{total}$ = 7.46/6.01/13.47 ms, spectral width = 5 kHz, average = 384, number of data point = 2048, total scan time = 32 min 10 sec). Unsuppressed water spectra were acquired with the same parameters as water suppressed spectra (excluding 8, averages; 50 sec, total scan time). Acquired *in vivo* raw data were analyzed in a fully-blind spectral process using LCModel with a simulated basis set containing 18 metabolites, as follows: alanine (Ala), aspartate (Asp), creatine (Cr), phosphocreatine (PCr), gamma-aminobutyric acid (GABA), glutamine (Gln), glutamate (Glu), glucose (Glc), glycine (Glyc), glycerophosphocholine (GPC), scyllo-inositol (sIns), myo-inositol (mIns), lactate (Lac), N-acetylaspartate (NAA), N-acetylaspartylglutamate (NAAG), phosphocholine (PCh), glutathione (GSH), and taurine (Tau). From the *in vivo* basis set, all signal intensities were processed with water scaling and eddy current correction, and obtained metabolite concentrations (µmol/g). All metabolites were fitted in the chemical shift range from 4.35 to 0.30 ppm. An independent t-test of the *in vivo* ^{1}H-MRS data, with the mean values of the metabolite concentrations by water references between the SSP and CNTL rats.

Results: Fig. 2 shows representative *in vivo* ^{1}H-MRS spectra acquired from the right dorsal hippocampal region. All metabolite signals were quantified using LCModel, with a simulated basis set. *In vivo* ^{1}H-MRS spectra were assigned the resulting 17 cerebral neurochemical signals. By visual inspection, the Glu signals were slightly higher in the SSP than in the CNTL rats (*in vivo* ^{1}H spectra in the CNTL rats are not shown). Sixteen metabolite signals from all *in vivo* ^{1}H spectra were visualized and analyzed below than in 20%SD (CRLB: Cramér–Rao-lower-bounds). Fig. 3 illustrates obtained cerebral metabolite concentrations that were quantified from the acquired *in vivo* ^{1}H-MRS spectra of the dorsal hippocampal region. Independent t-tests revealed significant differences of the cerebral metabolite concentrations between the two groups, indicating a significant stress-induced effect on quantified metabolite concentrations. The Glu concentrations were significantly lower in the dorsal hippocampal region of the SSP than in the CNTL rats (***; p = 0.004).

Discussion and Conclusion: In summary, we conducted *in vivo* ^{1}H-MRS in a rat model to quantitatively assess the influence of stress-induced sleep perturbation on cerebral neurochemical changes in the dorsal hippocampal region. Glu is the major mediator of excitatory synaptic neuronal transmission in the mammalian brain.[4] Previous studies have suggested that stress-related disorders are associated with perturbations in the metabolism of Glu.[5] Öngür and colleagues hypothesized that excessive glutamatergic neurotransmission driven by neuronal activity could be observed during insomnia associated with mania.[6] Moreover, Gln is converted to Glu by glutaminase and taken up by glial cells, where it is converted back to Gln by Gln synthetase (Glu/Gln cycle).[4] The alteration in Glu and/or Gln concentrations may reflect the dysfunction of glutamatergic synapses and neuronal-glial shuttle.[6] Therefore, combining our results with those from previous studies,[4-6] significantly lower Glu concentrations might reflect an alteration in the glutamate turnover rate in SSP, in comparison to CNTL rats, due to excessive glutamatergic neurotransmission driven by neuronal hyperactivity. We determined that significantly lower Glu signals in the hippocampus result from stress-induced sleep perturbation, which provides insights into neurochemical alterations and responses associated with psychiatric disorders.

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