Increased hippocampal glutamate after sleep deprivation in the pre-pubescent BALB/cJ mice: an in-vivo 1H MRS study

Manoj Kumar[1], Gaurav Verma[1], Ranjit Ittyerah[1], Steve Pickup[1], Edward S Brodkin[2], Ted Abel[3], and Harish Poptani[1]

[1]Radiology, University of Pennsylvania, Philadelphia, PA, United States, [2]Psychiatry, University of Pennsylvania, Philadelphia, PA, United States, [3]Biology, University of Pennsylvania, Philadelphia, PA, United States

Introduction: Neurotransmitters play an important role in maintaining functions, such as memory, learning, behavior and motor activity. Glutamate (Glu) is a major excitatory neurotransmitter associated with behavior, learning and memory functions, which are usually impaired in neuro-developmental and psychiatric disordersincluding autism-spectrum disorders (ASD), schizophrenia etc.[1].Behavioral, pharmacological and biochemical studies reveal dysfunction in the glutaminergic system in these disorders. Therefore, Glu and its regulatory molecules are considered as potential imaging and therapeutic targets for neuropsychiatric disorders[2].Sleep has important homeostatic functions, and sleep deprivationis a stressor that has consequences for the brain, as well as many body systems.Sleep deprivation is an efficient method totreat depressive symptomatology in neuropsychiatric disorders[3]. Previous study has been demonstrated relationship between sleepdeprivationsand changes in glutamate levels in the different regions of the rat brain[4].Changes in brain metabolites may be caused not only by the disease's progression or response to treatment, but also by physiological and behavioral changes[5]. The aim of this study was to use in-vivo^{1}H MRS to assess the effects of specific short-term sleep deprivation on the glutamatergic system in the BALB/cJ and C57BL/6J mice.

Materials and Methods:

Sleep Deprivation: Age/sex matched 30-day (pre-pubsescent) old BALB/cJ (ASD) mice[sleep-deprived (n=8) and controls (normal sleep pattern) (n=10)] and C57BL/6J [sleep deprived (n=6) and control (n=6) and were included in thisstudy.Sleep-deprived mousewere kept awake in theirhome cages by gentle stroking[6] to arouse themfrom sleep for 3 hrs andcontrols (non-sleep-deprived) mice were left undisturbed intheir home cages. In-vivo ^{1}H MRS was performed immediately after completion of 3hrs sleep-deprivation experiments and non-sleep-deprivedanimals were taken directly from undisturbed home cagesfor in-vivo MR spectroscopy.

Animal Preparation for MRI Scan: The isoflurane anesthesized mouse was placed in the coil after putting the animal in an in-house developed restraining device and the head wassecured in a nose cone and ear pins to minimize motion. Subdural needle electrodes, a respiratory pillow and a rectal thermister was placed and connected to a small animal vital signs device (SA Instruments, NY) to monitor ECG, respiration rate and core body temperature. During the scan, anesthesia was maintained with 1.5% isoflurane in air. During the scan body temperature was maintained at 37±1°C by blowing warm air through the magnet bore.

In-vivo ^{1}H MRS: In-vivo ^{1}H MRS was performed on a 9.4T horizontal bore scanner (Varian, Palo Alto, CA) equipped with 25 G/cm gradients. A 20mm i.d.quadrature birdcage coil (M2M, Cleveland, OH) was used for signal transmit and receive. Multi-slice spin echo T2-weighted images were acquired for planning the voxel. Single voxel^{1}HMRS was performed using a PRESS sequenceby placing a voxel of 2.5mmx1.5mmx1mm on the right hippocampus with following acquisition parameters: TR=3000ms, TE_1=13.89ms and TE_2 =10.01ms, number of averages=256, complex point=4096 and spectral width 4000Hz. Water suppression was performed using VAPOR technique. An unsuppressed water spectrum was also acquired (NT = 8) to compute metabolite to water ratios.

Tissue harvesting and Perchloric acid extraction: At the end of the in-vivo ^{1}HMRSstudy, each animal was immediately sacrificed with an overdose of anesthesia. Hippocampal tissueswas extracted from approximately the same location where in-vivo spectroscopic voxel was placed and tissue was frozen in liquid nitrogen and stored at -80^{0}C.Perchloric acid extraction and lyophilization wereperformed on these frozen brain tissues[7].

In-vitro high resolution ^{1}H MRS: Lyophilized samples were dissolved in 500µl of D20 with 0.5mMol TSP (as internal reference) and pD adjusted to 7.0 and ^{1}HMRS was performed at 11.7T, 55mm vertical bore spectrometer with following parameters: 45° pulse, TR=8s, SW=6,000Hz, NP=64K and NT=256) to confirm the in-vivo spectroscopic findings.

Spectroscopic data processing and data quantification: In-vitro spectroscopic data from extracts were analyzed using MestReNova (Mestrelab Research). In-vivo MRS data were analyzed using LC-model to measure the concentration [arbitrary units (AU), relative to water] of Glu, NAA, Cr and Cho.Independent t-test was performed between sleep deprived and non-sleep deprived mice for both BALBc/J and C57BL6 mice separately (Table 1).The major contribution of the peak at 2.36 ppm in-vivo is glutamate and it can be resolved from glutamine using the LC-Model analysis.

Table 1: In-vivo MRS data showing changes in metabolites in sleep deprived and control mice.

BALBc/J (ASD model)				
	Glu (Glx)	NAA	Cr	Cho
Non-sleep Deprived	11.33±1.40	5.46± 1.60	5.49±1.97	1.65±0.33
Sleep Deprived	15.24±2.13	5.67±1.04	3.22±1.76	2.10±0.34
p-value	**0.008**	0.82	0.17	0.08
C57BL6 (Healthy control mice)				
Non-sleep Deprived	12.68±2.23	7.44±1.95	4.69±2.55	1.65±0.56
Sleep Deprived	13.78±1.78	5.82±2.27	3.58±2.64	1.82±0.33
p-value	0.37	0.22	0.48	0.55

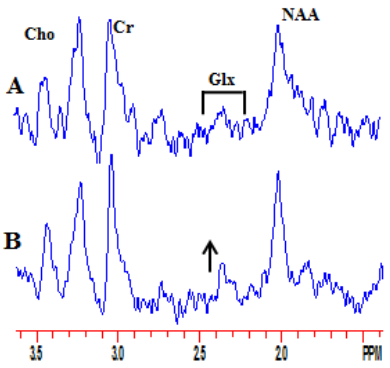

Fig. 1: Demonstrating in-vivo spectra from control (A) and sleep deprived (B) BALB/cJ mice. Arrow indicatessignificantly increased Glx (Glutamate) in sleep deprived BALB/cJ mice. No significant changes in other metabolites were observed in the two groups.

Results: We observed a significant increase in Glu in pre-pubsescent sleep deprived BALB/cJ mice. At pre-pubescence, these animals demonstrate reduced sociability[4]. In comparison, the more social C57BL/6J mice did not demonstrate any changes in the Glu concentrations after 3 hrs of sleep deprivation. These in-vivo results were confirmed by in-vitro high resolution NMR of the extracted brain tissue where the resonance of glutamate and glutamine can be clearly resolved.

Discussion: In–vivo and ex-vivo spectroscopy demonstrating significantly increased Glu in sleep deprived BALB/cJ mice compared to controls. Previous in-vitro studies on rat brain extracts also reported increased glutamate from the hippocampusand thalamus[4]. The increased in Glu only in sleep deprived BALB/cJ mice, relative to the more social C57BL/6J mice leads to a possibility that alterations in glutaminergic system may have a causal effect on social behavior. It is also possible that the less social BALB/cJ mice are more susceptible to sleep-induced changes. Future studies with longer duration of sleep deprivation or at different times during the circadian cycle may help in establishing this relationship.

References: [1] Moghaddam B and Javitt D. Neuropsychopharmacology. 2012;37:4-15. [2] Choudhury PR, et al., Pharmacol Biochem Behav. 2012;100:841-49. [3] McEwen BS. Metabolism. 2006;55:S20-23.[4] Cortese BM, et al., Physiol Behav. 2010;99:445-50[5] Brodkin ES. Behav Brain Res. 2007; 176: 53-65. [6] Urrila AS, et al., Psychiatry Res. 2006;147:41-46.[7] Ledoux, L, et. al, Brain Res. 1996;735:108-18.

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