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

Manoj Kumar1, Gaurav Verma2, Ranjit Ittyerah3, Steve Pickup1, Edward S Brodkin2, Ted Abel3, and Harish Poptani3

1Radiology, University of Pennsylvania, Philadelphia, PA, United States; 2Psychiatry, University of Pennsylvania, Philadelphia, PA, United States; 3Biology, 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 disorders including autism-spectrum disorders (ASD), schizophrenia etc.3,4. Behavioral, pharmacological and biochemical studies reveal dysfunction in the glutamergic system in these disorders. Therefore, Glu and its regulatory molecules are considered as potential imaging and therapeutic targets for neuropsychiatric disorders.5 Sleep has important homeostatic functions, and sleep deprivation is a stressor that has consequences for the brain, as well as many body systems. Sleep deprivation is an efficient method to treat depressive psychopathology in neuropsychiatric disorders6. Previous studies have shown a demonstrated relationship between sleep deprivation and changes in glutamate levels in the different regions of the rat brain.7 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.8 The aim of this study was to use in-vivo 1H 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-pubescent) 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) were included in this study. Sleep-deprived mice were kept awake in their home cages by gentle stroking3 to arouse them for sleep for 3 hrs and controls (non-sleep-deprived) mice were left undisturbed in their home cages. In-vivo 1H MRS was performed immediately after completion of 3hrs sleep-deprivation experiments and non-sleep-deprived animals were taken directly from undisturbed home cages for in-vivo MR spectroscopy.

Animal Preparation for MRI Scan: The isolurane anesthetized mouse was placed in the coil after putting the animal in an in-house developed restraining device and the head was secured in a nose cone and ear pins to minimize motion. Subdural needle electrodes, a respiratory pillow and a rectal thermoster 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 1H MRS: In-vivo 1H 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 voxel1H MRS was performed using a PRESS sequence placing a voxel of 2.5mmx1.5mmx1mm on the right hippocampus with following acquisition parameters: TR=3000ms, TE1=13.89ms and TE2=10.01ms, number of averages=256, complex point=4096 and spectral width 4000Hz. Water suppression was performed using VAPOR technique. An unsuppressed water signal was also acquired (NT = 8) to compute metabolite to water ratios.

Tissue harvesting and perchloric acid extraction: At the end of the in-vivo 1H MRS study, each animal was immediately sacrificed with an overdose of anesthesia. Hippocampal tissues were extracted from approximately the same location where in-vivo spectroscopic voxel was placed and tissue was frozen in liquid nitrogen and stored at -80°C. Perchloric acid extraction and lyophilization were performed on these frozen brain tissues.

In-vitro high resolution 1H MRS: Lyophilized samples were dissolved in 500μl of D2O with 0.5mMol TSP (as internal reference) and pH adjusted to 7.0 and 1H MRS 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 BALB/cJ 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 or in-vitro high resolution NMR of the extracted brain tissue where the resonance of glutamate and glutamine can be clearly resolved.

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

<table>
<thead>
<tr>
<th>Age/sex matched 30-day (pre-pubescent) old BALB/cJ (ASD) mice</th>
<th>Sleep Deprived</th>
<th>Control</th>
</tr>
</thead>
<tbody>
<tr>
<td>Glu (Glx)</td>
<td>11.3±1.40</td>
<td>12.68±2.23</td>
</tr>
<tr>
<td>NAA</td>
<td>5.46±1.60</td>
<td>5.82±2.27</td>
</tr>
<tr>
<td>Cr</td>
<td>5.49±1.97</td>
<td>5.67±1.04</td>
</tr>
<tr>
<td>Cho</td>
<td>1.65±0.33</td>
<td>7.44±1.95</td>
</tr>
<tr>
<td>p-value</td>
<td>0.008</td>
<td>0.17</td>
</tr>
</tbody>
</table>

Results: We observed a significant increase in Glu in pre-pubescent sleep deprived BALB/cJ mice. At pre-pubescence, these animals demonstrate reduced sociability. 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-vivo 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-vivo studies on rat brain extracts also reported increased glutamate from the hippocampus and thalamus. The increased in Glu only in sleep deprived BALB/cJ mice, relative to the more social C57BL/6J mice leads to the possibility that alterations in glutamergic 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.


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