Decrease of glutamate in the hippocampus of the *fmr1* knockout mouse during myelingenesis detected by *in vivo* ^{1}H MRS

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Introduction:

Fragile X syndrome (FXS) is the most common form of inherited mental retardation. Men with FXS generally have low IQ while women have borderline to normal IQ [1]. Neuronal abnormalities include dense and immature dendrites and behavioral abnormalities are common in FXS. This genetic disease is caused by mutational insertion of tri-nucleotide repeats in the Fmr1 gene which codes for Fragile X mental retardation protein (FMRP). FMRP is known to be involved in RNA processing and regulation of protein synthesis at synapses that could affect regulation of N-methyl-D-aspartic acid (NMDA), α-amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid (AMPA) and metabotropic glutamate (mGluR) receptors [1]. The *fmr1* knockout mice are an ideal model for understanding the alterations in brain using non-invasive methods such as ^{1}H MRS and allow for non-invasive monitoring of brain structure and metabolic integrity of the brain. We focused on the hippocampal region of the mouse as previous *ex vivo* studies have shown increased protein synthesis [2] and changes in excitatory synaptic response in the hippocampus of the knockout mice [3,4]. Specifically, we determined the differences in the metabolites within the hippocampus between the *fmr1* knockout mice and wild type C57Bl6J mice using *in vivo* ^{1}H MRS.

Methods and Materials:

fmr1 knockout mice (JAX B6.129P2-fmr^{1tmlCgr} mice, Jackson Laboratory, Bar Harbor, Maine) were obtained from a colony at UMB. Four male *fmr1* knockout and eight wild type (JAX C57Bl6J) mice were used in this study. All experiments were performed on a Bruker Biospec 7.0 Tesla 30 cm horizontal bore scanner using Paravision 5.1 software. A four channel Bruker ^{1}H surface array coil was used as the receiver and a Bruker 72 mm linear-volume coil as the transmitter. Proton density-weighted MR images were obtained using a 2D rapid acquisition with relaxation enhancement (RARE) sequence (TR/TE=5500/9.5 ms) for anatomic reference. A point-resolved spectroscopy (PRESS) pulse sequence (TR/TE=2500/20 ms) was used for data acquisition from a 2 x 4 x 2 mm^3 voxel that covered all layers of the hippocampus (Figure 1). Data were acquired at 18, 21 and 30 days of age for each mouse. For each spectrum, 300 acquisitions were averaged for a total of 13 min. At all times during the experiment, the mouse was under 1-1.5% isoflurane anesthesia and 1 L/min oxygen administration. Respiratory monitoring was performed and the animal temperature was maintained at 36-37°C during the entire experiment. ^{1}H MRS data was fitted using the LCModel package. A paired two-tail Student t-test was performed between the mean metabolite concentrations to tCr ratio at each time point for to assess the difference between the two groups of mice.

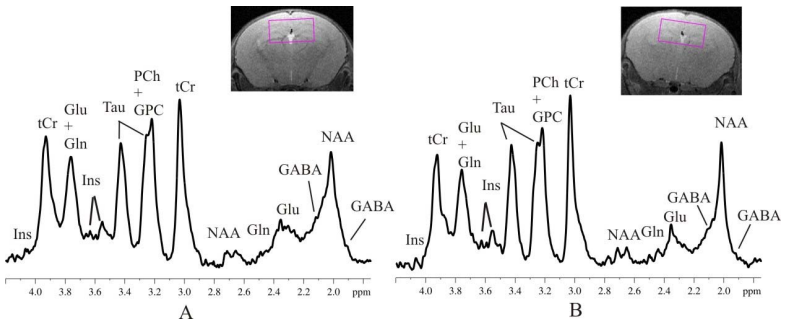

Figure: *In vivo* ^{1}H MRS on hippocampus region at 21 day old of A) a wild type mouse and B) a *fmr1* knockout mouse.

Results:

An example ^{1}H MR spectra from a wild type and a *fmr1* knockout mouse are demonstrated in the Figure. An increase in hippocampal glutamate (20-40%) was found for the *fmr1* knockout at 21 days ($p<0.01$) compared to the wild type. There were no changes in glutamine and GABA metabolites at 18, 21 or 30 days of age. To determine the balance between excitability or inhibitory responses in the brain, we calculated glutamate to GABA ratio between *fmr1* and wild type mice and found the relative concentration approaching significance at 18 days ($p<0.06$) but not at 21 days. Also we observed an increase (20-40%) in taurine ($p<0.01$) and a decrease (50-60%) in insositol ($p<0.05$) at 18 and 21 days between *fmr1* knockout mouse compared to wild type.

Discussion:

Currently one ^{1}H MRS study is available on patients with FXS which reports a decrease of choline in the prefrontal cortex [7]. It was the first report on the use of *in vivo* ^{1}H MRS to characterize FXS. Our study is the first to characterize changes in brain metabolites in the animal model of FXS in the hippocampus. An increase in glutamate at 21 days in *fmr1* knockout mouse may indicate an increase in mGluR activation and altered excitatory neurotransmission previously described in *fmr1* knockout models in *ex vivo* studies [2]. The near significance of glutamate to GABA ratio also suggests an increase of excitatory neuro-transmission and possibly a decrease of inhibtory transmission, similar to findings in the cortical pathways [5]. Treatments approved to date for human use have focused on decreasing typical phenotypes such as hyperactivity, mood disorders, anxiety [1] and normalizing dendritic spines [8]. Several pre-clinical studies have shown the effectiveness of mGluR antagonist methyl-6-(phenylethynyl)pyridine (MPEP) in normalizing FXS phenotype in mice [6] including one Phase 1 trial in humans [9]. Given the differences that we observe in glutamate and GABA activity, our study indicates that *fmr1* knockout model is a viable model to identify secondary alterations and study treatment effects. *In vivo* ^{1}H MRS could provide important information about effects of therapy on metabolite changes in the brain of FXS patients.

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References:

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