

Imaging of Glutamate Alterations in Alzheimer's Disease

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Introduction: Glutamate (Glu) is the major free amino acid present in the brain and functions as an excitatory neurotransmitter. The central role of Glu in learning, memory and cognition is well reported¹ and has been shown to decrease in Alzheimer's disease (AD) pathology^{2,3}. Magnetic resonance imaging (MRI) and proton MR spectroscopy (¹HMRS) are widely used in studying the structural and biochemical changes in AD brain during disease progression^{2,4}. Previous ¹HMRS studies on human brain have depicted progressive decrease in hippocampal Glu concentration from cognitive control to mild cognitive impairment (MCI) to full blown AD⁵. Although, ¹HMRS has been widely used to monitor the Glu changes in AD it suffers from poor spatial resolution and long acquisition time. Recently, mapping Glu in healthy brain was performed using the technique known as chemical exchange saturation transfer (CEST) by exploiting its amine proton exchange with the bulk water (GluCEST)⁵. Here, we report the mapping of the Glu distribution in the brain of APP-PS1 transgenic mouse model of AD as well as age matched wild type (WT) mice at high resolution by utilizing GluCEST technique. The potential implication of GluCEST in detecting early AD pathology is discussed.

Materials and Methods: Animal Preparation: The Institutional Animal Care and Use Committees of the University of Pennsylvania approved experimental protocols. Six APP-PS1 mice and six WT mice age spanning from 18 to 20 months were used in this study (from Wyeth Research). Mice were transferred to a 9.4T horizontal bore small animal MR scanner (Varian, Palo Alto, CA) and placed in a 20-mm diameter commercial quadrature proton coil (m2m Imaging Corp., Cleveland, OH). Animals were kept under anesthesia (1.5% isoflurane in 1 liters/min oxygen) and their body temperature maintained with the air generated and blowing through a heater (SA Instruments, Inc., Stony Brook, NY). **Imaging Protocols:** GluCEST imaging of the mouse brain was performed using a custom-programmed segmented RF spoiled gradient echo (GRE) readout pulse sequence with a frequency selective continuous wave saturation preparation pulse. The sequence parameters were: field of view = 20x20 mm², slice thickness = 2 mm, flip angle = 15 degree, GRE readout TR = 6.2 ms (128 segments), TE = 2.9 ms, matrix size = 128x128. For every 8 s one saturation pulse and 128 acquisition segments were applied. CEST images were collected using a 1 second saturation pulse at peak B₁ of 250 Hz and frequencies ranging ±5 ppm from bulk water in step size of 0.2 ppm. B₁ and B₀ field maps were also acquired and used to correct the GluCEST contrast as described previously⁵. CEST imaging was performed on two different brain slices. The total imaging time was around 30 min. **¹H Magnetic Resonance Spectroscopy:** Single voxel spectra (SVS) were performed with stimulated echo acquisition mode (STEAM) using a vendor (Varian) provided pulse sequence with the following parameters: voxel size = 3.5 mm x 3.5 mm x 2 mm (Voxel volume 24.5 μL), spectral width = 4 kHz, number of points = 4006, averages = 264, TE = 8 ms, Tm = 7 ms, and TR = 5 s. Water suppression was achieved using the variable pulse power and optimized relaxation delays method (VAPOR). Glu concentration relative to total creatine (tCr) was measured using LC model⁶.

Results and Discussion: The brain anatomical images and corresponding GluCEST maps and MRS spectra from WT and APP-PS1 mice are shown in figures 1 (A-C) and 2 (A-C). The GluCEST maps illustrate the regional distribution of Glu in different regions of brain both in WT and AD mouse (Figs. 1B & 2B). The higher GluCEST contrast was observed in the gray matter compared to the white matter, which is due to the difference in the Glu concentration and is consistent with the previous studies on rat and human brain⁷. The mean GluCEST contrast from the WT and APP-PS1 mice brain over the chosen voxel was 26.4±1.6 % and 19.1±1.9 %. ¹HMRS spectra show decreased Glu peak amplitude in APP-PS1 mice compared to WT mice (arrows, Figs. 1C & 2C). The mean Glu/tCr in WT and APP-PS1 mice was 1.58±0.13 and 1.12±0.08, respectively. Comparative analysis showed ~28% decreased GluCEST (p<0.01) contrast and ~29% decrease (p<0.01) in Glu/tCr in APP-PS1 mice brain than in that of WT mice over the chosen voxel. The z-spectra and MTR asymmetry curve obtained from WT and APP-PS1 mouse are shown in figure 3A and 3B, respectively. The 3 ppm line on the MTR asymmetry curves corresponds to GluCEST. The bar graphs show the average GluCEST contrast and Glu/tCr both in WT and APP-PS1 mice (Figs. 4A & 4B). The plot in figure 4C shows the correlation between Glu/tCr measured and GluCEST contrast. An excellent positive correlation (R²=0.91) was observed with a slope of ~15% GluCEST per Glu/tCr ratio. Hippocampus is the primary structure associated with the early loss of the pyramidal neurons and their synapses in AD pathology, which control the learning and cognitive function. The GluCEST maps of the brain slice showing hippocampus regions both in WT and AD mice were also obtained (Figs. 5A-C). In AD mice brain, an average of ~31% decreased GluCEST contrast was found in hippocampus compared to WT. The other factor that may contribute to GluCEST is magnetization transfer effect from bound water pool. No significant change in MTR contrast was observed in AD mice compared to WT mice (data not shown). The findings of this preliminary study suggest that using GluCEST it is feasible to obtain *in vivo* high-resolution maps of altered regional Glu concentration in AD pathology. The large changes of GluCEST observed in this study from fully developed AD model coupled with the previously published MRS results from AD and MCI patients suggest that it has sufficient dynamic range to detect changes from MCI stage of the disease. Given that these molecular changes are associated with incipient stages of the disease, this method has the potential to detect changes before structural alterations in the brain.

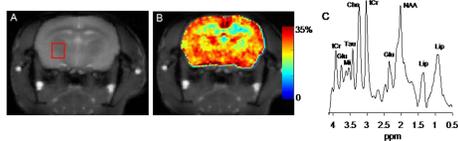


Figure 1. A, The anatomical brain image from a wild type mouse. B, The corresponding GluCEST map. C, The ¹HMRS spectra from the voxel placed in (A) shows the major brain metabolites. The glutamate peaks are labeled at 2.3 and 3.75 ppm.

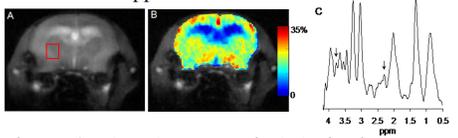


Figure 2. A, The anatomical brain image, B, The corresponding GluCEST map of a APP-PS1 mouse. Figure C shows the ¹HMRS spectra for the voxel placed in (A). Decreased GluCEST contrast along with concomitant decrease in glutamate was observed compared to WT.

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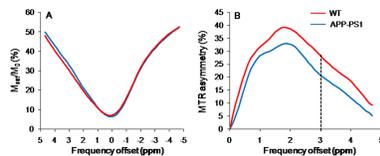


Figure 3. z-spectra (A) and MTR asymmetry curves (B) from WT and APP-PS1 mice for the voxel shown in figures 1A and 2A. The dotted line at 3ppm in MTR asymmetry curves reflects the GluCEST contrast

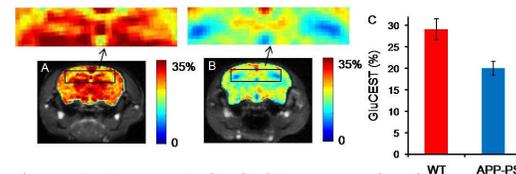


Figure 4. The bar graphs show the mean GluCEST contrast (A) and Glu/tCr from WT and AD mice. The graph (C) shows a strong positive correlation between GluCEST and Glu/tCr.

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Figure 5. Decreased GluCEST contrast in hippocampus was observed in APP-PS1 mice compared to WT mice. The hippocampus regions are shown in rectangular box. The bar graphs show the mean hippocampus GluCEST contrast in WT and AD mice respectively.