Sub-regional Hippocampus Glutamate Changes in a Mouse Model of Tau Pathology Measured by GluCEST

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BACKGROUND: Synapse loss is the main correlate of cognitive defecits in AD, rather than pathologic protein [1]. Glutametergic synapse loss is among the earliest symptoms of disease found in AD brain tissue. Magnetic resonance spectroscopy (MRS) has shown that glutamate (Glu) and N-acetyl-aspartate (NAA) were decreased in the hippocampus of AD patients compared to healthy subjects [2]. In this study, we measured glutamate levels *in vivo* in the brain of the PS19 mouse model of tau pathology [3] using a novel imaging technique called glutamate chemical exchange saturation transfer (GluCEST) [4]. We hypothesize that regional changes in glutamate levels will correlate with symptoms of pathology including synapse and neuron loss.

METHODS: The PS19 mouse model is a transgenic mouse which overexpresses mutated human tau, at P301S, a common mutation in FTDP-17 [3]. All animal studies were approved by the university's IACUC. Glutamate concentration was measured *in vivo* in WT (n=8, mean age=17.9 months), and PS19 mice (n=9, mean age=18.3 months) by ^{1}H Magnetic Resonance Spectroscopy (^{1}H MRS), and GluCEST. Imaging was performed on a 9.4T spectrometer (Varian Inc., Palo Alto, CA). MRS was performed using the PRESS pulse sequence (TR/TE 3000/14ms, 384 averages), with VAPOR water suppression. GluCEST imaging was performed using a custom-programmed RF spoiled gradient echo readout pulse sequence, with a frequency selective continuous wave saturation preparation pulse. CEST images were collected using a 1 second saturation pulse at peak B_1 of 250 Hz for the frequencies ± 2.4 – 3.6 with steps of 0.2ppm from water resonance. B_1 and B_0 maps were acquired to correct inhomogeneities. All images and spectroscopic data were processed as described previously [4]. Regions of interest (ROIs) were segmented from T2-weighted images. Sub-regions of the hippocampus were segmented based on a threshold from GluCEST maps: above 30% as dentate gyrus (DG), and the mid-range values as the *cornu ammonis* (CA). Immunohistochemistry (IHC) was analyzed for syanpse loss (synaptophysin), and neuron loss (NeuN).

RESULTS:

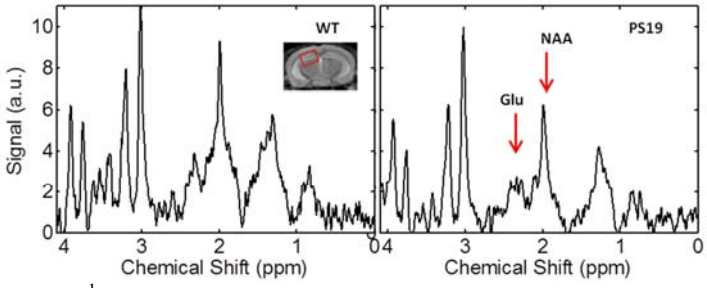

Figure 1. ^{1}H MRS acquired from the entire hippocampus region shows decreased NAA and Glu, which is consistent with glutamate levels measured by GluCEST. Spectroscopy does not allow for sub-regional analysis of these metabolites.

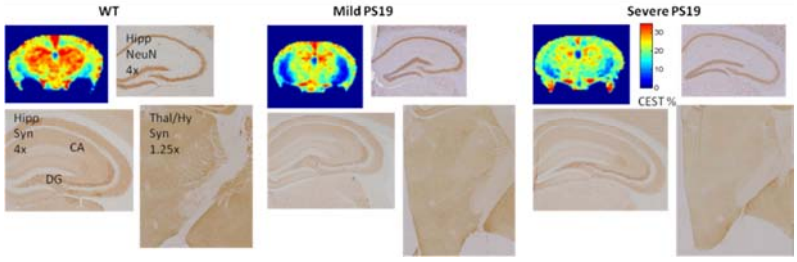

Figure 2. GluCEST maps clearly show lower glutamate levels throughout the PS19 mouse brain compared to WT. IHC reveals no significant neuron loss in PS19 mouse hippocampus. Rather, synapse density is consistently diminished in PS19 mice, both in the hippocampus CA sub-region and the thalamus/hypo-thalamus regions. In thePS19 DG sub-region of the hippocampus, synapse staining remains strong.

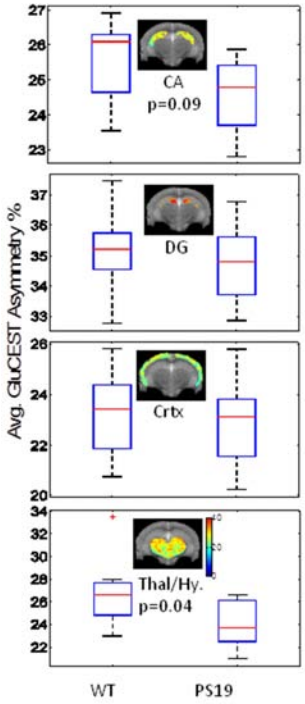

Figure 3. Average GluCEST contrast of the thalamus/hypo-thalamus region is significantly decreased in PS19 mice (23.9 ± 1.04 % vs. 26.8 ± 1.60 %, p≤0.05, mean ± std). Interestingly, glutamate is decreased in the CA sub-region of the hippocampus of PS19 mice, yet maintained in the DG.

CONCLUSIONS: GluCEST imaging allows for sub-regional analysis of glutamate in the hippocampus of the Tauopathy mouse brain, an advantage when compared to conventional spectroscopic methods. Glutamate levels are decreased throughout the pathologic brain, which correlates with synapse loss in these regions. We have shown *in vivo* that glutamate levels and synapse density remain high in the DG sub-region of the hippocampus, where there is known to be a high level of neuronal turnover [5].

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