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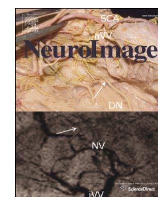

In vivo measurement of glutamate loss is associated with synapse loss in a mouse model of tauopathy

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ABSTRACT

Glutamate is the primary excitatory neurotransmitter in the brain, and is implicated in neurodegenerative diseases such as Alzheimer's disease (AD) and several other tauopathies. The current method for measuring glutamate *in vivo* is proton magnetic resonance spectroscopy (^{1}H MRS), although it has poor spatial resolution and weak sensitivity to glutamate changes. In this study, we sought to measure the effect of tau pathology on glutamate levels throughout the brain of a mouse model of tauopathy using a novel magnetic resonance imaging (MRI) technique. We employed glutamate chemical exchange saturation transfer (GluCEST) imaging, which has been previously validated as a complimentary method for measuring glutamate levels with several important advantages over conventional ^{1}H MRS. We hypothesized that the regional changes in glutamate levels would correlate with histological measurements of pathology including pathological tau, synapse and neuron loss. Imaging and spectroscopy were carried out on tau transgenic mice with the P301S mutation (PS19, $n = 9$) and their wild-type littermates (WT, $n = 8$), followed by immunohistochemistry of their brain tissue. GluCEST imaging resolution allowed for sub-hippocampal analysis of glutamate. Glutamate was significantly decreased by 29% in the CA sub-region of the PS19 hippocampus, and by 15% in the thalamus, where synapse loss was also measured. Glutamate levels and synapse density remained high in the dentate gyrus sub-region of the hippocampus, where neurogenesis is known to occur. The further development of GluCEST imaging for preclinical applications will be valuable, as therapies are being tested in mouse models of tauopathy.

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Introduction

Tauopathy is a classification of neurodegenerative diseases with more than 20 phenotypes, which include varying symptoms of impaired motor function and dementia (Ballatore et al., 2007; Hutton et al., 1998). The defining pathology of tauopathy is the presence of neurofibrillary tangles (NFTs) composed of hyper-phosphorylated tau protein (HPtau). Tau is a microtubule binding protein where the extent of phosphorylation at multiple sites regulates its binding to microtubules (Alonso et al., 2001). Pathological tau is found in the form of fibrillar aggregates, which accumulate in the perikarya and dendritic spines of hippocampal neurons and, among other consequences, disrupt the function of neurotransmitters (Dubois et al., 2007). Adequate *in vivo* diagnoses and therapies for tauopathy are being actively studied, primarily for Alzheimer's disease (AD), and also for corticobasal degeneration (CBD), progressive supranuclear palsy (PSP), amyotrophic lateral sclerosis/parkinsonism–dementia complex, Down's syndrome, and several frontotemporal dementias (Lee et al., 2001).

Alterations in glutamate levels have been observed in several tauopathies. The neurotransmitter pool of glutamate is affected by decreased glutamate transporters and receptors in the hippocampus and cortex of AD patients compared to healthy controls (Greenamyre and Maragos, 1993). Glutamate transporters were also found to be associated with phosphorylated tau in neurofibrillary tangles in patients with AD, PSP, and CBD (Sasaki et al., 2009). The metabolic pool of glutamate is also affected in tauopathies. The TCA-cycle synthesizes glutamate from alpha-ketoglutarate, which is in a state of hypometabolism in early stages of AD (Mosconi, 2005; Nilsen et al., 2013; Sancheti et al., 2014) and in PSP (Albers et al., 2000).

Extracellular glutamate can be monitored using microdialysis methods (Minkeviciene et al., 2008); however to measure brain glutamate concentration noninvasively *in vivo*, the current standard method

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is proton magnetic resonance spectroscopy (^{1}H MRS). Using ^{1}H MRS, Rupsingh et al. reported a decrease in glutamate in the hippocampus of AD patients compared with healthy controls (Rupsingh et al., 2011). Despite the biochemical evidence of reduced glutamate in human tauopathy cases, transgenic mouse models of tauopathy have been only sparsely studied using ^{1}H MRS. Mouse models of tauopathy with the P301L mutation have shown decreased glutamate levels in the hippocampus (Yang et al., 2011) and in hippocampal extracts (Nilsen et al., 2013) by ^{1}H MRS. However, the spatial distribution of glutamate changes is impossible to measure using MRS because of its limited spatial resolution.

We recently described an MRI technique that is sensitive to glutamate levels in the brain: glutamate chemical exchange saturation transfer (GluCEST) imaging (Cai et al., 2012). GluCEST is complimentary to MRS in that it provides a measure proportional to glutamate concentration ([Glu]), at physiologic pH, but it is superior in several important factors. GluCEST imaging has higher spatial resolution than spectroscopic methods. Also, smaller changes in glutamate levels can be measured by GluCEST. Compared to the glutamate signal at 2.35 ppm measured by ^{1}H MRS, the measurable GluCEST effect has two orders of magnitude greater signal. In other words, a higher dynamic range of glutamate levels can be measured using GluCEST imaging compared to single-voxel spectroscopy. This proves to be a valuable tool in diseases where glutamate changes are subtle, as may be the case for early stages of dementia. In a recent publication, our group has demonstrated the feasibility of measuring glutamate changes using GluCEST and validated the measurement with ^{1}H MRS in a mouse model of AD with amyloid-beta pathology (Haris et al., 2013).

In this study, we have used GluCEST imaging as well as ^{1}H MRS to study the consequence of tau pathology on glutamate in a P301S mouse model. *In vivo* measures of glutamate are correlated with histological measurements of tau burden, including the severity of pathological tau, neuron loss, and synapse loss. Given the translational opportunities of GluCEST, our findings in a mouse model of tauopathy have immediate potential application to clinical studies.

Methods

PS19 transgenic mouse model of tauopathy

The mouse model of tauopathy studied here was the PS19 line of the P301S transgenic mouse, overexpressing the human P301S mutant tau found in FTDP-17 patients, developed by Yoshiyama et al. (Yoshiyama et al., 2007). The first sign of tau pathology in this mouse model is defective axonal transport, followed by synapse loss and hyperphosphorylated tau accumulation at presynaptic terminals. As the animals age, pathological tau progresses along the perforant pathway, from the entorhinal cortex into the hippocampus and pre-frontal cortex, while severe neuron loss is apparent at later stages of disease (Hurtado et al., 2010). Behavioral studies show decreased ability in spatial learning with the progression of disease (Brunden et al., 2010). In this study, we have imaged aged PS19 mice (n = 9, mean age = 20.7 months), and their age-matched wild-type (WT) littermates (n = 8, mean age = 19.0 months). This study was approved by the university's IACUC. Note that the onset of pathology in this generation of mice is later than originally published (see current generation (Zhang et al., 2012).

MRS acquisition

All spectroscopy and imaging studies were performed on a 9.4 T using a 30 cm horizontal bore magnet fitted with an 11 cm gradient insert and interfaced to a Varian spectrometer (Agilent Technologies Inc., Santa Clara, CA), with a vendor-supplied, mouse volume coil (M2M Imaging Corp., Cleveland, OH). Mice were anesthetized using isoflurane (1–2% in 1–2 L/min oxygen) for the duration of the scan. During the study animals were kept at 37 °C using a heater and air-pump to blow hot air into the bore of the magnet. These methods were approved by the IACUC of the University Pennsylvania.

^{1}H MRS was performed on WT (n = 8) and PS19 (n = 7) mice using the PRESS pulse sequence (TR/TE = 3000/14 ms, spectral width = 4 kHz, number of points = 4006). The variable pulse power and optimized relaxation delays (VAPOR) water suppression technique was used to acquire a water-suppressed spectrum (averages = 384), and another spectrum was acquired without water suppression to obtain the water reference signal for normalization (averages = 16). Unsuppressed water spectra had line widths of 20 Hz or less after localized shimming. Total acquisition time for spectroscopy was about 20 min. Spectra were acquired from a voxel localized in the hippocampus ($2 \times 2.5 \times 3$ mm^3).

The integrals of the peaks of glutamate ([Glu]) and other metabolites of interest, including *N*-acetyl-aspartate (NAA), were calculated from spectra using in-house written software (MATLAB 7.9.0 R2009b). We are reporting the ratio of the integral of the metabolites with that of creatine. Manual baseline correction and phasing were performed. Metabolite peak locations and widths were identified manually, and combined with a pre-determined set of macromolecule peaks. The peak-fitting routine performs a nonlinear, least squares fitting of Lorentzian peaks to the spectra (MATLAB "lsqcurvefit"). The ratio of the integrals of [Glu] and [NAA] peaks to the internal standard of total creatine (tCr) is reported.

GluCEST imaging

GluCEST (glutamate chemical exchange saturation transfer) image contrast is based on amine protons of glutamate that are in chemical exchange with protons on bulk water. The amine protons are saturated by application of a frequency-selective radio frequency (RF) pulse. As the saturated magnetization (zero net magnetization) of amine protons is exchanged with bulk water protons, bulk water MRI signal is reduced in proportion to the concentration of glutamate.

In the brain, the signal can be affected by direct water saturation and background magnetization transfer effect. To account for these effects, two images are obtained, one with saturation at the resonance frequency of amine exchanging spins (+3 ppm downfield from water for glutamate amine protons) and a second image with equal frequency offset on the other side of the bulk water peak (-3 ppm). The CEST effect of the amine spins is given by the difference in the asymmetry ratio (Eq. (1)).

$$GluCEST_{asym(\Delta\omega=3ppm)} = \frac{M_{sat(-\Delta\omega)} - M_{sat(\Delta\omega)}}{M_{sat(-\Delta\omega)}} \quad (1)$$

The M_{sat} ($\pm\Delta\omega$) are the magnetizations obtained with saturation at a '+' and'−' offset to the water resonance; $\Delta\omega$ is equivalent to the resonance offset of the exchanging spins.

There are several important considerations in the pulse sequence design. Amine protons on glutamate resonate at 3 ppm offset downfield from water ($\Delta\omega$ ~ 7200 rad s^{-1} at 9.4 T), with an exchange rate (k ~ 3000 s^{-1}) in the slow to intermediate regime ($\Delta\omega > k$). We have implemented a saturation pulse of 1 second duration (4 Hanning pulses at 250 ms each, with a 4 μs inter-pulse-delay), with an amplitude B_{1rms} of 5.9 uT, which has been optimized in order to adequately saturate the amine protons on glutamate (Haris et al., 2013). A segmented, spoiled GRE readout is used, with 2 shots for each offset frequency. Each shot consists of one saturation pulse and a 64 segment readout (segment TR/TE = 6.7/3.4 ms). The time between shots is set to 8 s to allow for T1 recovery. The final image parameters are: matrix size = 128 × 128, FOV = 20 × 20 mm, slice thickness = 1 mm, voxel size = 0.16 × 0.16 × 1 mm^3. GluCEST maps were acquired from one

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