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Longitudinal imaging reveals sub-hippocampal dynamics in glutamate levels associated with histopathologic events in a mouse model of tauopathy and healthy mice

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Abstract

Tauopathies are neurodegenerative disorders characterized by abnormal intracellular aggregates of tau protein, and include Alzheimer's disease, corticobasal degeneration, frontotemporal dementia, and traumatic brain injury. Glutamate metabolism is altered in neurodegenerative disorders manifesting in higher or lower concentrations of glutamate, its transporters or receptors. Previously, glutamate chemical exchange saturation transfer (GluCEST) magnetic resonance imaging (MRI) demonstrated that glutamate levels are reduced in regions of synapse loss in the hippocampus of a mouse model of late-stage tauopathy. We performed a longitudinal GluCEST imaging experiment paired with a cross-sectional study of histologic markers of tauopathy to determine whether 1) early GluCEST changes are associated with synapse loss before volume loss occurs in the hippocampus, and whether 2) subhippocampal dynamics in GluCEST are associated with histopathologic events related to glutamate alterations in tauopathy. Live imaging of the hippocampus in three serial slices was performed without exogenous contrast agents, and subregions were segmented based on a k-means cluster model. Subregions of the hippocampus were analyzed (*cornu ammonis* CA1, CA3, dentate gyrus, and ventricle) in order to associate local MRI-observable changes in glutamate with histological measures of glial cell proliferation (GFAP), synapse density (synaptophysin, VGlut1) and glutamate receptor (NMDA-NR1) levels. Early differences in GluCEST between healthy and tauopathy mice were measured in the CA1 and

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I. Disclosure Statement for Authors

The authors have no conflicts of interest to declare.

DG subregions (30% reduction, $p = 0.001$). Synapse density was also significantly reduced in every subregion of the hippocampus in tauopathy mice by 6 months. Volume was not significantly reduced in any subregion until 13 months. Further, a gradient in glutamate levels was observed *in vivo* along hippocampal axes that became polarized as tauopathy progressed. Dynamics in hippocampal glutamate levels throughout lifetime were most closely correlated with combined changes in synaptophysin and GFAP, indicating that GluCEST imaging may be a surrogate marker of glutamate concentration in glial cells and at the synaptic level.

B. Introduction

A variety of neurodegenerative disorders are classified as tauopathies due to an abnormal presence of intracellular aggregates of tau protein in the brain. Alzheimer's disease (AD) is the most well known tauopathy. Other diseases in which tau pathology is the most dominant feature include frontotemporal dementia with parkinsonism linked to chromosome 17 (FTDP-17), corticobasal degeneration (CBD), progressive supranuclear palsy (PSP), and Pick's disease (PiD) (Lee et al., 2001; Thibodeau et al., 2009). Recently, research interest in tauopathies has further intensified with the discovery that chronic traumatic encephalopathy (CTE) and traumatic brain injury (TBI), which particularly affect athletes of contact sports and military personnel exposed to repeated explosions, is a progressive tauopathy (Lakis et al., 2013; McKee et al., 2009).

The earliest symptoms of tauopathy are found at the synaptic level (Selkoe, 2002). In mouse models of tauopathy, structural and functional abnormalities of synapses in the hippocampus are measurable earlier (Yoshiyama et al., 2007) and independent of neurofibrillary tangles (NFTs) (Rocher et al., 2010). Synapse loss in cortical tissue of AD patients is the closest histologic correlate to cognitive deficits (Terry et al., 1991), and to the severity of cognitive deficits (DeKosky and Scheff, 1990). In several mouse models of tauopathy, early synapse loss has likewise been shown to correlate with deficits in learning and memory tasks: a P301L human tau mouse model (Maurin et al., 2014), a regulated P301L expression model rTG4510 (Hoover et al., 2011), and a pro-aggregate human tau model (Hochgräfe et al., 2013). With the possibility that these cognitive deficits are reversible (Hochgräfe et al., 2013; Sydow et al., 2011; Van der Jeugd et al., 2012), development of an *in vivo* diagnostic biomarker which correlates with synapse loss is highly desirable for testing potential therapies.

A novel magnetic resonance imaging (MRI) method, glutamate chemical exchange saturation transfer (GluCEST) imaging, is able to measure glutamate levels *in vivo* (Cai et al., 2012). The GluCEST technique magnetically labels amine protons residing on endogenous glutamate that exchange with bulk water to produce contrast proportional to the concentration of total free glutamate (5–15mM per kg wet weight brain tissue, Schousboe 1981). With such high concentrations in the brain, GluCEST is able to image glutamate levels with high sensitivity and spatial resolution without the use of contrast agents. While GluCEST imaging has shown deficits in mouse models of AD (Haris et al., 2013) and tauopathy (Crescenzi et al., 2014), the sensitivity of GluCEST to changes in glutamate levels throughout the course of disease has not yet been examined.

There are two main sources of neuronal glutamate: synthesized during the tricarboxylic acid cycle, and derived from glutamine from astrocytes as part of the glutamine-glutamate cycle. Reduced synthesis of glutamate and glutamine were measured in hippocampal tissue of a rat model of AD with advanced pathology (Nilsen et al., 2014a). In younger rats with AD, before amyloid beta pathology is detectable, reduced glutamate was measured in the hippocampus (Nilsen et al., 2014b). These age effects were primarily determined from excised tissue using complimentary techniques of MR spectroscopy, which motivates the use of *in vivo* GluCEST imaging to determine longitudinal changes in glutamate levels.

It is also important to consider that glutamate functions as the primary excitatory neurotransmitter in the central nervous system (CNS). A portion of neuronal glutamate is packaged into neurotransmitter vesicles to be transported to the presynapses via vesicular glutamate transporter protein (VGlut, Bellocchio et al., 2000). VGlut1 was discovered to be reduced in AD cortical tissue (Kirvell et al., 2006), and found to correlate with cognitive impairment scores (Kashani et al., 2008). VGlut1 was also reduced in the perforant pathway of patients with cognitive impairment (Robinson et al., 2014); however, in patients a decade younger (mean age 88.8 yrs) with cognitive impairment, VGlut1 was found to be elevated over healthy controls in the mid-frontal gyrus (Bell et al., 2007).

Once released into the synaptic cleft, glutamate doesn't remain long in the extracellular space, or else it would become excitotoxic (Hertz et al., 1999). Glutamate is actively transported into astrocytes by the glutamate transporter protein (GLT-1), also known as excitatory amino acid transporter protein (EAAT). Astrocytes are the location of the glutamine-glutamate cycle, by which three quarters of glutamate is ultimately recycled and stored in the form of glutamine (Benjamin and Quastel, 1975; Westergaard et al., 1995). In the hippocampus and mid-frontal gyrus from sporadic AD patients, reduced expression and protein levels of EAAT1 and EAAT2 were observed local to amyloid beta plaques (Jacob et al., 2007). This reduction may be due to their association with phosphorylated tau localized to NFTs in AD, PSP, and CBD patients (EAAT2, Sasaki et al., 2009).

Postsynapses of other neurons and glia accept extracellular glutamate via glutamatergic receptors. Early autoradiography and immunohistochemistry (IHC) studies in AD tissue have shown reduced NMDA (N-methyl D-aspartate) binding in the cortex (Greenamyre and Young, 1989; Jansen, 1990; Penney et al., 1990), reduced AMPA (α -amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid) binding in the hippocampus (Dewar et al., 1991) and reduced AMPA receptors in the entorhinal cortex (Yasuda et al., 1995) and hippocampus (Aronica et al., 1997). Reduced expression levels of genes encoding NMDA and AMPA receptors were also measured in the AD hippocampus (Jacob et al., 2007).

What is known about changes in glutamate levels *in vivo* at the subhippocampal level is primarily from long-term potentiation (LTP) studies. Glutamate levels are an indicator of synaptic growth during LTP (Dolphin et al., 1982), and may be an indicator of synaptic dysfunction in neurodegenerative disease (Clare et al., 2011; Gong and Lippa, 2010). LTP is dependent upon NMDA receptors in the *cornu ammonis* hippocampal subfield CA1 and the dentate gyrus (DG), however synaptic transmission does not depend on NMDA receptors in the mossy fibers of CA3 (Lynch et al., 2004; Nicoll and Schmitz, 2005). LTP was found to

be impaired in the CA1 and not the CA3 of a mouse model of tauopathy exhibiting cognitive deficits (Chong et al., 2011). In contrast, LTP is enhanced in the DG of young (2 to 3 months) tauopathy mice (Boekhoorn et al., 2006; Pennanen et al., 2006). An effective *in vivo* surrogate biomarker of synapse loss based on glutamate levels would correlate with the subhippocampal location and dynamics of synapse loss.

Previously GluCEST deficits have been measured in locations of severe synapse loss in a tauopathy mouse model (Crescenzi et al., 2014). However, this study focused on the late stage of disease after synapse loss and volume loss had already occurred. The primary goal of the current study, therefore, was to measure longitudinal dynamics in subhippocampal glutamate levels, from early to late stage pathology, in order to determine when glutamate changes occur in relation to synapse and volume loss. Subregions of the hippocampus were analyzed in order to associate local changes in GluCEST contrast with histological markers. A variety of cell-type specific antibodies (GFAP), a general synapse marker (synaptophysin) and presynaptic glutamate transporter (VGlut1) and postsynaptic receptor (NMDA-NR1) antibodies were applied to tissue from age-matched littermates. This study was driven by two main hypotheses: 1) decreased GluCEST signal will occur when synapse loss is measurable, and before volume loss; and 2) GluCEST signal will be sensitive to dynamics in glutamate levels in specific subregions of the hippocampus, and associated with histologic events as disease progresses in PS19 mice. The findings will aid our interpretation of GluCEST image contrast and its potential utility to image onset of synapse loss and disease progression in preclinical tauopathy.

C. Methods

C1. Experimental Design

The IACUC of the University of Pennsylvania approved all experiments. These experiments were performed on a PS19 mouse model of tauopathy that overexpresses mutated human-tau (T34 isoform, 1N4R) at P301S (first described by Yoshiyama et al., 2007, specific generation used here described by Hurtado et al., 2010). This is a mutation found in FTDP-17 patients. In mice this mutation causes decreased microtubule density and impaired LTP in the CA1 by 3 months (Yoshiyama et al., 2007) and impaired cognition in a Barnes maze test by 6 months (Brunden et al., 2010). Hyper-phosphorylated tau inclusions develop progressively, with eventual neurofibrillary tangle formation (see stages by Hurtado et al., 2010). There is an insignificant and minor drop-off in the Kaplan–Meier Survival Curve between 6 and 7 months (Hurtado et al., 2010). Male mice were housed in a humidity controlled room ~22°C under a 12 hour light/ dark cycle with *ad libitum* access to food and water. PS19 mice and their WT littermates were imaged at successive time points, in two cohorts: Cohort 1 WT (n=6) and PS19 (n=6) at 3, 7 and 13 months of age; and Cohort 2 WT (n=3) and PS19 (n=6) at 9 and 13 months of age. In order to correlate *in vivo* imaging data to histological measures as PS19 mice age, from the same generation of littermates raised under identical housing conditions, sectioned tissue was retrospectively obtained from the CNDR library at available ages: 3 months (n=4 WT, n=4 PS19), 6 months (n=6 PS19), and 9 months (n=6 PS19). Histology was also performed on the mice that were imaged (13 months of age).

C2. MRI preparation

In vivo imaging was performed on a 9.4T small-animal spectrometer (Agilent Technologies Inc., Santa Clara, CA). Mice were anesthetized using isoflurane gas (induced with 2% isoflurane, maintained with 1.5–2% isoflurane mixed with oxygen at 2 L/min), and secured to a body-bed inside a 20mm diameter volume coil (M2M Imaging Corp., Cleveland, OH). Mice were prepared on a warming table, and body temperature was monitored during imaging using an anal temperature probe and maintained at 37°C using forced hot-air inside the bore of the magnet (M2M Imaging Corp., S2PAM software). Respiratory rate was monitored using a pressure-pillow underneath the chest, connected to a fiber-optic readout, and maintained free breathing between 40–80bpm.

C3. Glutamate Chemical Exchange Saturation Transfer (GluCEST) MRI

The GluCEST MRI technique provides high-resolution maps of glutamate levels in the brain by magnetically labeling endogenous amine protons of free glutamate. Amine protons undergo chemical exchange with bulk-water protons, transferring their magnetic saturation state that reduces the water signal. This is the chemical exchange saturation transfer (CEST) effect. The reduction in water signal is measured as the asymmetry between images acquired with saturation at the resonant frequency of amine protons of glutamate (3 ppm offset from water) and with saturation equidistant from water (−3ppm), according to the equation:

$$GluCEST_{asym} = \frac{M_{sat(-3ppm)} - M_{sat(3ppm)}}{M_{sat(-3ppm)}} \quad \text{Eqn. (1)}$$

In equation 1, M_{sat} is the signal acquired after a saturation pulse. Subtraction of these images and normalization provides a map that is proportional to the CEST effect, with minor contributions from direct saturation and magnetization transfer effects on water. GluCEST asymmetry % (CEST %) is proportional to the concentration of total free glutamate within the voxel. Contributions from other labile amine and amide protons contribute to <30% of the GluCEST asymmetry. Detailed descriptions of the GluCEST technique can be found in Cai et al., 2012, Haris et al., 2013, and Crescenzi et al., 2014.

The pulse sequence is comprised of a frequency selective saturation preparation pulse ($B_{1rms} = 5.87 \mu T$ and duration of 1s as four pulses of 250 ms duration each) applied before a spoiled gradient echo readout (effective TR/TE = 6.6/3.3 ms). The whole preparation/acquisition combination is repeated every 8s. Four averages were acquired at each saturation frequency from $\pm 2.5 - 3.5$ ppm offset from water with a step size of 0.25 ppm. This range allows for B_0 inhomogeneity to be corrected up to 0.5 ppm. B_0 and B_1 maps were also acquired and used to correct the GluCEST contrast as described previously (Crescenzi et al., 2014). GluCEST, B_0 , and B_1 maps were acquired from 3 consecutive slices (1mm thick each, in-plane resolution $156 \mu m^2$) spanning the hippocampus from rostral to caudal (Figure 1i). The center slice position was chosen 2.3 mm rostral to the Lambda marker, which was identified on a sagittal scout image. The total scan time including GluCEST, B_0 and B_1 mapping for each slice was approximately 15 min. Anatomical T₂-weighted images were acquired with identical resolution and position (multi-slice fast spin-echo, TR/TE = 3000/36 ms, 2 averages, matrix = 128×128 , FOV = 20mm, slice thickness = 1 mm).

C4. Image segmentation by k-means cluster analysis

We were interested in observing the GluCEST signal from four anatomical subregions of the hippocampus: the DG, the CA1 and CA3, and the adjacent fourth ventricle containing cerebral spinal fluid (CSF). It is not possible to delineate these subregions from a T₂-weighted *in vivo* MRI (Figure 1i). Although high spatial resolution atlases exist, both for MRI (Johnson et al., 2010) and histology (Adler et al., 2014), these methods require registration with exact *ex vivo* tissue, which cannot be obtained in a longitudinal study.

Therefore, we first manually segmented the hippocampus proper from the T₂-weighted image (Figure 1i). The outline of the hippocampus can be adequately delineated in each slice: rostral (“R”), middle (“M”, mid), and caudal (“C”). The hippocampal mask was applied to the corresponding GluCEST map (Figure 1ii–iii).

A secondary segmentation was then applied to each hippocampus using the k-means clustering algorithm (Matlab script by Jose Vicente Manjon Herrera, 2005). This algorithm uses a statistical method to set a threshold between high, medium, and low GluCEST values with respect to each hippocampus slice (Figure 1iv). The k-means method has been used to segment gray matter, white matter, and CSF regions of interest (ROIs) from *in vivo* anatomical MRIs of the brain (Leung et al., 2015; Vemuri et al., 2008). We adapted this approach to segment three “clusters” or final subhippocampal ROIs from each hippocampus slice based on the GluCEST contrast. Examples of segmented clusters from three slices of the mouse hippocampus are shown in Figure 1v. The locations of subhippocampal ROIs within each slice roughly fall along the dorsal-ventral axis. Therefore segmented regions are labeled within each slice: dorsal “D” in red, intermediate “I” in green, and ventral “V” in blue (Figure 1v–vii). This nomenclature was motivated by the segmentation presented by Dong et al., 2009 based on gene-expression patterns within the hippocampus.

The ROIs qualitatively correspond to the location of hippocampal subregions of interest, thereby extending the cluster analysis of GluCEST to anatomical subregions of the hippocampus. In Figure 1vi the relevant subregions (CA1, DG, CA3) are outlined on hippocampus atlases and colored according to their corresponding ROI. In total, 9 subregions were segmented from the hippocampus of each mouse.

The average GluCEST value in each region was averaged across the group of WT and PS19 mice at each age. Additionally, the volume of each region was measured as the number of pixels normalized to the total hippocampus volume. Group averages from the 9 regions were arranged in a grid, and are referenced according to slice position [rostral (R), middle (M), caudal (C)] and ROI location [dorsal (D), intermediate (I), ventral (V)]. An anatomical model of corresponding hippocampal subregions is provided in Figure 1vii for reference.

C5. Immunohistochemistry

All mice brains were prepared using standard methods of transcardial perfusion/fixation. Briefly, mice were deeply anesthetized using a mixture of xylazine, ketamine, and acepromazine (100:10:1 mg/kg, according to their weight). Intracardiac perfusion was then performed using phosphate-buffered saline (0.01M), followed by 20 mL of 10% neutral

buffered Formalin. Brains were then dissected and stored overnight at 4°C in 10% NBF, and embedded in paraffin the following day. Paraffin blocks were sliced 6 µm thick.

Several antibodies were applied to sectioned brain tissue. AT8 monoclonal antibody was utilized to depict phosphorylated tau at residues Ser202/Thr205 (applied primary antibody concentration 1:7500, generated at the CNDR), developed with a horseradish peroxidase developing system (BioGenex, Hyderabad, AP India) with diaminobenzidine peroxidase substrate as the chromogen (DAB, ImmPACT(TM), Vector Laboratories, Burlingame, CA, USA) and counterstained using hematoxylin. Synaptophysin (syn., Milipore Corporation, Bedford, MA, USA, Mab38 anti-rabbit, 1:500) was applied for presynaptic terminals, developed using the ABC system (VECTASTAIN(R), Vector Laboratories). VGlut1 (presynaptic vesicular glutamate transporter protein, Synaptic Systems, Catalog #135304, anti-guinea pig, 1:5000) received microwave pre-treatment for 15 minutes at 100°C in citrate buffer (Vector Antigen Unmasking Solution, Vector Laboratories), and was developed using the ABC system. To quantify glial cell proliferation, antibody to GFAP (glial fibrillary acidic protein, generated at the CNDR, 1:2000) was applied, developed using the ABC system, and counterstained with hematoxylin. An antibody for NMDA glutamate receptor subunit 1 (NR1, Abcam, Cambridge, MA, USA, 1:5000, with citrate pretreatment) was applied, developed using the BioGenex system, and counterstained with hematoxylin.

Binding of the following antibodies were quantified in the mid-hippocampus corresponding to the imaged slice position: synaptophysin, VGlut1, and GFAP. Slides were chosen in the mid-hippocampus based on a visual match to Bregma -2.18 anatomy (see Figure 1vi, middle atlas section). Sections were discarded if damaged or incorrect anatomy was determined after immunostaining. Immunostained PS19 tissue at 13 months was not quantified due to the atrophic hippocampus in aged PS19 mice, so that ROI selection was not possible. Actual slice positions of stained tissue sections used for quantification were evaluated by an independent observer (M.D.B), by comparing atlas anatomy with slices stained for synaptophysin. Note that alternative immunogens were applied to subsequent tissue sections. An average Bregma position of -2.05 mm, standard deviation 0.8 mm, standard error of the mean 0.12 mm, was determined. The standard deviation is within the comparative MRI slice thickness of 1 mm.

Stained slides were scanned using an automatic slide scanner (Lamina Scanner, PerkinElmer, Waltham, MA). Scanned slides were viewed using CaseViewer (v1.3.0.41885, Histech, Budapest, Hungary), and snapshots were taken at 10x magnification. ROIs were segmented by hand in the dentate gyrus polymorph and granule cell layer, and all laminae of the CA1 and CA3 (see Figure 5b for example ROIs). Images were thresholded to the same level for all mice (ImageJ, NIH). The amount of immunostaining was measured as the percentage of area occupied (% AOC) by positive staining within the ROI. GFAP immunostaining was distinguished from hematoxylin by the color deconvolution plug-in (Ruifrok 2001) for ImageJ using the hematoxylin and DAB optical densities (ODs) provided; the hematoxylin image was used for ROI identification, and the chromagen image was thresholded before measuring the %AOC.

Slides immunostained with NR1 were quantified in the caudal hippocampus (Bregma -3.18, see Figure 1vi third atlas section) because tissue from the middle hippocampus was limited after optimization of previously described immunostaining protocols. ROIs were outlined in the DG, CA1, and CA3. Positive chromagen signal was measured in the NR1 stain by identification and averaging of red, blue, green (RGB) OD values from five random NR1 positive neurons for color deconvolution of signal from background and counterstain. A minimum OD threshold was applied to measure the percentage of area occupied (% AOC) by NR1 positive neurons, confirmed for accuracy by visual inspection. Note that caudal tissue from 6 month (n=4) and 9 month (n=1) PS19 mice was combined to gain statistical power.

C6. Statistical analyses

To determine the effects of age and genotype on GluCEST and volume results, linear mixed effect models (LMM) for repeated measures were applied. The models combined all data across ROIs and considered random intercepts for mouse, region and slice in addition to a random slope for age. Fixed effects included indicators for the 9 clusters (including 3 regions in each of 3 slices), age, genotype and an interaction between age and genotype. Age was modeled as a categorical variable if departures from linear trends were observed. A significant age by genotype interaction indicated differences in the outcome over time by genotype. A significance level of $p < 0.05$ was considered statistically significant and Chi-square values are reported wherever necessary. Higher order interactions with region and slice indicators were included in the models to test for group difference by cluster. Scheffe's method was used to account for multiple testing. GluCEST data from Cohort 1 acquired at 7 months and Cohort 2 at 9 months were combined to gain statistical power. Data from Cohort 2 at 3 months was not acquired, however LMM was chosen for its flexibility in handling missing data.

Student's two-tailed t-tests were also performed to determine significant differences between group averages at various timepoints, assuming unequal variance. For GluCEST and volume data, a significance level of $p < 0.001$ was determined after Bonferroni correction for 3 slices, 4 timepoints, and 3 regions. For histology data that was quantified in a single slice, comparisons were made between PS19 mice against WT mice at 3 months; a significance level of $p < 0.004$ was determined after Bonferroni correction for 3 regions and 4 time-points. A significance level of $p < 0.01$ was also reported but is not significant in the strictest sense. Additional t-tests were performed among PS19 mice between age-groups for each region, with a significance level of $p < 0.025$ after Bonferroni correction between two timepoints. Error bars express the standard error of the mean (SEM).

D. Results

D1. Longitudinal glutamate imaging in subregions of the hippocampus

Representative GluCEST maps from the hippocampus of a WT and PS19 mouse are shown at each age in Figure 2. GluCEST contrast is clearly not homogeneous within the hippocampus. Several obvious features can be observed. The caudal slice generally has higher GluCEST asymmetry than the rostral slice. A region of high GluCEST is maintained

throughout lifetime in the dorsal hippocampus. The ventral hippocampus has reduced GluCEST contrast and is enlarged in the PS19 mouse compared to WT.

Figure 3 illustrates that a spatial gradient is observed in glutamate levels in the mouse hippocampus. GluCEST values are highest in the most caudal-dorsal regions of the hippocampus, and are lowest in the more rostral-ventral regions. Specifically, the caudal-dorsal (C-D) region has the highest GluCEST value at each age, and the rostral-ventral (R-V) region has the lowest GluCEST value in both WT and PS19 mice.

In comparison between PS19 and WT mice, at 3 months of age the average GluCEST asymmetry is elevated throughout the hippocampus in PS19 mice (insignificant trend, positive values in Figure 3c). In all regions of PS19 mice, GluCEST decreases from 3 to 7 months. In WT mice GluCEST increases in all regions from 3 to 7 months. The combination of these effects produces the earliest and greatest significant difference in GluCEST between WT and PS19 mice at 7 months in the middle-dorsal (M-D) region (30% difference, $p = 0.001$, starred in Figure 3c). After adjusting for multiple comparisons, the change in GluCEST from ages 3 to 7 months was significantly different by genotype (LMM analysis, Scheffe p -value=0.012).

After 7 months, GluCEST in WT mice progressively diminishes but remains higher than PS19 mice in all regions except the M-D. Rather, at 13 months in the PS19 M-D region, GluCEST is elevated over WT levels (positive value in Figure 3c). Several other dorsal regions of the PS19 hippocampus show increases in GluCEST compared to earlier time-points in the PS19 cohort: the M-D and R-D regions increase from 7 to 9 months and from 9 to 13 months, the C-D and M-I regions increase from 9 to 13 months (Figure 3). LMM analysis confirms that within PS19 mice, there was a significant difference in GluCEST over time by slice and region (Chi-square ($df=8$) = 68.0, $p = 0.001$).

In contrast, the ventral and majority of intermediate regions of the PS19 hippocampus progressively decrease from 3 to 13 months. GluCEST in the M-V region of PS19 mice is significantly lower than WT at 9 months ($p = 0.001$, starred in Figure 3c). By 13 months GluCEST is significantly reduced in all ventral regions of PS19 mice compared to WT, and the C-I region. According to the LMM analysis, there is a significant difference in GluCEST over time by genotype and region adjusting for slice (Chi-square ($df=6$) = 46.98, $p = 0.001$), and by genotype and slice adjusting for region (Chi-square ($df=6$) = 23.87, $p = 0.001$).

D2. Proportional volume changes in subregions of the hippocampus

Longitudinal volume changes are represented as proportion of the total hippocampus, and subregion results are arranged in a grid (Figure 4) following the scheme of Figure 1vii. The volume of the ventral hippocampus of PS19 mice generally follows that of WT mice until 13 months. The greatest and most significant difference in volume is measured in the M-V region at 13 months (58% higher compared to WT, $p = 0.001$). The C-V region also occupies a larger volume of the PS19 hippocampus than WT by 13 months ($p = 0.001$).

Volume of the intermediate PS19 hippocampus decreases throughout lifetime below WT volume. The volume of the C-I and M-I regions reach a significant reduction by 13 months

($p < 0.001$). Concurrently, the dorsal PS19 hippocampus increases in volume through 9 months. The volume of the C-D region remains elevated over WT at 13 months ($p < 0.001$). Although volume differences at 13 months are significant, the LMM analysis considers longitudinal volume changes and reports that volume is not significantly different over time by genotype (Chi-square ($df=1$) = 0, $p > 0.99$), adjusting for region and slice.

D3. Pathological events in PS19 tauopathy

Tau pathology in this cohort of PS19 mice is presented in Supplemental Figure 1 (Figure S1). At 3 months of age, hyper-phosphorylated tau (by AT8 immunostaining) is present in the first layer of the caudal cortex, the entorhinal cortex, and mossy fibers of the CA3 from rostral to caudal (Figure S1a). As PS19 mice age to 6 months, AT8 pathology is present in all layers of the hippocampus, and has infiltrated deeper layers of the cortex, the entorhinal cortex, and amygdala (Figure S1b). By 9 months tau pathology is present throughout the hippocampus, and tissue degeneration is observed in some mice at this age (Figure S1c). Additional affected regions include the thalamus (Figure S1c rostral iii) and frontal cortex (not shown). By 13 months, volume loss is notable with an expanded fourth ventricle throughout the hippocampus (Figure S1d).

The middle section was used for subsequent immunohistochemistry considering that the largest, earliest deficits in GluCEST were measured in the middle hippocampus. The earliest significant difference in synapse density was measured at 6 months in PS19 mice compared to WT mice at 3 months in the CA1 (Figure S2) and CA3 subregions of the hippocampus ($p < 0.004$, Figure 5d, Table 1a). Synapse density continues to decrease in all subregions of the hippocampus of PS19 mice out to 9 months.

VGlut1, a marker of glutamatergic presynapses, reveals interesting dynamics as PS19 mice age. At 3 months VGlut1 is reduced in all subregions of the PS19 hippocampus compared to WT (Figure S3, Table 1b). By 6 months VGlut1 immunostaining %AOC is higher than 3 and 13 month WT values. In the DG of PS19 mice at 6 months, there is a trend of increased VGlut1 compared to the 3 month PS19 cohort ($p=0.02$, Figure S3c). By 9 months VGlut1 is again reduced from WT in all subregions ($p < 0.01$ in CA3, Table 1b). VGlut1 immunostaining is also reduced in WT mice throughout lifetime (Table 1b), though to a lesser extent.

GFAP immunostaining increases with aging in both healthy and tauopathy mice. Figure 6a shows more affected regions in the PS19 brain including the hippocampus, amygdala, thalamus, and cortex compared to WT. By 9 months, GFAP was significantly increased in all subregions of the PS19 hippocampus compared to WT ($p < 0.004$, Figure 6c, Table 1c).

Immunostaining of glutamate receptor NR1 reveals fewer NR1-positive cells in the PS19 CA1 (Figure S4). This trend was observed in all subregions of 3 month PS19 mice compared to WT (Table 1d). Further reductions were measured by 6 to 9 months in all subregions ($p=0.06$ compared to WT in the CA1, Table 1d).

E. Discussion

E1. Glutamate gradient measured *in vivo* by GluCEST MRI

To facilitate interpretation of the cluster analysis segmentation of ROIs in terms of hippocampal subregions, an anatomical model is provided in Figure 1vii. Dorsal ROIs correspond anatomically to the DG and may include contributions from CA1, although they remain more dorsal than CA3. The intermediate ROIs therefore include the CA3. In the caudal slice, the CA1 extends from the dorsal to ventral hippocampus in young mice, and may therefore be represented by the dorsal, intermediate, and ventral ROIs. The ventral ROIs contain subfields of the CA in younger mice, and transition to CSF in older mice as tissue is lost and the ventricle expands. These regions are subject to partial volume effects between adjacent anatomies.

The purpose of displaying GluCEST averages in a grid, along the rostral-caudal and dorsal-ventral axes, is to visualize the gradient in glutamate levels measured here *in vivo*. Anatomically this could also be interpreted as a glutamate gradient along the septo-temporal axis, also known as the functional “long” axis of the hippocampus (Strange et al., 2014).

Numerous studies have reported gradients along these axes within the hippocampus in terms of gene expression (Fanselow and Dong, 2010; Thompson et al., 2008), connectivity (Risold and Swanson, 1996), electrophysiology (Staresina et al., 2012), and spatial processing functions (Kjelstrup et al., 2008).

Gradients of neuron activation and neurogenesis have also been observed along these axes within the rodent hippocampus. Neurons in the most dorsal hippocampus are highly active even without a stimulating memory task (Snyder et al., 2011). There is also a greater density of newborn neurons in the dorsal DG compared to ventral regions of the DG in young (Snyder et al., 2009) and old rats (Snyder et al., 2011). These findings are consistent with our observation that the highest glutamate levels measured by GluCEST imaging were observed in the dorsal DG from rostral to caudal (Figure 3).

While the discovery of a glutamate gradient is largely confirmatory in this study, we have demonstrated that GluCEST imaging is capable of measuring this gradient *in vivo* with high spatial resolution and coverage of the entire hippocampus. The GluCEST MRI method may be used as a complimentary technique to a variety of biological measurements in the mouse hippocampus.

E2. Reduced GluCEST and synaptic density are measured before volume loss in the PS19 mouse model of tauopathy

Differences in hippocampal GluCEST levels between WT and PS19 mice occur at each age measured in this study. The earliest and greatest difference in GluCEST between PS19 and WT mice was measured at 7 months of age in the middle-dorsal region (30% reduced from WT, $p = 0.001$). LMMs report a significant difference in GluCEST over time by genotype (Chi-square ($df=3$) = 13.37, $p=0.0039$), however volume is not significantly different over time by genotype (Chi-square ($df=1$) = 0, $p>0.99$), adjusting for region and slice. This is

likely due to the fact that hippocampal volume loss was not significantly different until 13 months in PS19 mice compared to their WT littermates.

The only other study to date to report volume loss and reduced CEST contrast in a tau mouse model with hippocampal tau tangles uses complimentary techniques to show significant total hippocampal volume loss and significant differences in total hippocampal CEST contrast (Wells et al., 2015). Our findings show that reduced GluCEST occurs not only earlier than volume loss, but also in a different location than volume changes within the subhippocampal fields. A shift in location of GluCEST deficits is observed in Figure 3c, where differences shift from the more dorsal-caudal regions at 7 months to the more ventral-caudal regions by 13 months (red highlight Figure 3c).

In vivo GluCEST results reflect a deficit in glutamate levels which is concurrent with reduced synapse density in the same subregions. Early deficits in GluCEST at 7 months occur throughout the entire middle ($p = 0.001$) and caudal hippocampus slices. Synapse density was also significantly reduced in the middle hippocampus in PS19 mice at 6 months ($p = 0.004$ in CA1 and CA3). The caudal CA1 also shows reduced synapse density by 6 months (Figure S2).

This is in contrast to lower GluCEST in the ventral hippocampus of older PS19 mice. By 13 months, the C-V region is filled with CSF which has low glutamate concentration compared to brain tissue. This is a result of hippocampal volume loss and ventricle volume increase. Volume changes in subhippocampal regions normalized by total hippocampal volume should be interpreted as a proportional volume measurement. The steep changes in PS19 volume at 13 months are driven by a large increase in the ventral hippocampus subregion, which proportionally decreases dorsal and intermediate regions such that the majority of the hippocampal space is ventricle by 13 months. Prior to 13 months there is an increasing trend in the volume of the dorsal PS19 hippocampus in all the rostral, middle, and caudal slices. This can be understood as a polarization of glutamate distribution in the hippocampus such that the highest GluCEST values become concentrated in the dorsal hippocampus that appears to grow (see GluCEST maps in Figure 2 from a 13 month PS19 mouse).

E3. Early glutamate elevation may correspond with excitotoxicity

Early signs of tau pathology were observed in both the GluCEST measurement and synaptophysin immunostaining. At 3 months there was a trend of higher glutamate levels in every subregion of the PS19 hippocampus compared to WT (positive difference Figure 3c), which may be relevant in the pathway of excitotoxicity. At this age hyperphosphorylated tau pathology was only present in the CA3 mossy fibers, whereas the trend in synapse density was lower in all subregions analyzed. A potential explanation for this early state of widespread elevated glutamate, which associates with synapse deficits in the absence of phosphorylated tau, could be excitotoxicity. A higher GluCEST signal of 1–2% measured in 3 month old PS19 hippocampus corresponds to an elevation of glutamate by approximately 2–3 mM/L (Cai et al., 2012). This is more than sufficient to induce excitotoxicity (Nicholls and Budd, 1998). Additional experiments are needed to confirm this relationship; however, this finding demonstrates the potential for GluCEST imaging to measure excitotoxicity *in vivo*.

Throughout the hippocampus, elevated GluCEST levels at 3 months (Figure 3) preceded an increase in VGlut1 levels at 6 months in PS19 mice (Figure S3). Another study found a similar trend of elevated VGlut1 at 5 months in a P301L mouse model by immunoblotting of hippocampus tissue compared to WT mice (Hunsberger et al., 2015). Elevated VGlut1 coincided with decreased expression of GLT-1, a transporter involved in clearing glutamate from the extracellular space (Hunsberger et al., 2015), which may be a response to excitotoxicity. We have also shown a progressive decrease in NR1, an NMDA receptor, which resides on the post-synapse (Figure S4, Table 1d). Direct evidence in cultured cortical neurons shows that reduced expression of NMDA receptors may be protective, in that NMDA receptor activation is required for tau-induced cell death (Amadoro et al., 2006). In a tauopathy mouse model, reduced levels of AMPA receptor GluR1 were found in post-synaptic dendritic spines, where pathologic tau accumulates preferentially over the pre-synapse (Hoover et al., 2010). This occurred at a similar age to VGlut1 dynamics, 4.5 months, before loss of the spine structure itself.

This trend may translate to early stages of clinical neurodegeneration. Elevated presynaptic density via VGlut1 was shown in the mid-frontal gyrus of patients with early stages of mild cognitive impairment (Bell et al., 2007). Early elevations in glutamate represent an important therapeutic window for intervention. GluCEST MRI has the potential to show early dynamics in glutamate levels during neurodegeneration *in vivo* and would be a useful tool to monitor onset and therapeutic response.

E4. GluCEST as a surrogate marker of combined trends in synaptophysin and GFAP

As WT and PS19 mice age, glutamate levels have complex dynamics in distinct subregions of the hippocampus. We suspect there are both developmental effects and pathologic effects creating these dynamics. An unexpected finding of this study is a ubiquitous increase in hippocampal GluCEST levels in WT mice, measured longitudinally from 3 to 7 months. One process that may elevate glutamate levels during lifetime is proliferation of glial cells. It is known that GFAP immunostaining increases during healthy aging in mice (Kohama et al., 1995), rhesus macaques (Haley et al., 2010), and humans (David et al., 1997).

Tauopathy mice do not exhibit a longitudinal increase in hippocampal glutamate at 7 months likely due to impaired synapse plasticity during development. Tau protein is highly functional during neonatal development, although microtubule associated protein tau (MAPT) gene knock out mice have no overt phenotype and develop normally (Denk and Wade-Martins 2009) due to compensation by other MAP proteins. The mouse model used here incorporates transgenic mutant human tau. The primary result is formation of hyperphosphorylated tau (Figure S1), which reduces the number of microtubules and reduces fast axonal transport (Zhang et al., 2012), possibly disrupting development by means of reduced transmission and synapse plasticity. In tau transgenic mice in general, cognitive deficits are not measurable until hyperphosphorylated tau is present (Denk and Wade-Martins 2009). Cognitive deficits are significant in the PS19 mouse model by 6 months (Brunden et al., 2010), the age that mice display significantly lower synapse density and thereafter GluCEST deficits throughout the hippocampus.

Increased GluCEST is observed later at 9 months in the PS19 hippocampus in the dorsal ROIs only, from rostral and middle slices, and further at 13 months in all dorsal ROIs and the M-I region (Figure 3b). These regions correspond to the DG and CA1 from rostral to caudal. This is concurrent with more than 15-fold increase in GFAP immunostaining in each subregion of the PS19 hippocampus at 9 months (Figure 6c). Astrocyte activity may reflect an effort to clear extracellular glutamate to be recycled through the glutamate-glutamine cycle. However, while the neurotransmitter pool of glutamate in the extracellular space can be up to 100 μ M during transmission, the metabolic pool of glutamate as an energy source to cells is likely a more significant component of the GluCEST signal. The largest concentration of glutamate occurs in neurons (10–15mM, 7-fold greater than that of other metabolites), and there is a substantial contribution from glial cells (6–10mM) (McKenna et al., 2007).

The GluCEST signal is directly proportional to the amount of total free glutamate in the brain, and may reflect dynamics in neuronal and glial glutamate metabolism. For instance, GluCEST levels in the caudal-ventral hippocampus progressively decrease in PS19 mice (red highlight Figure 3c), associated with greater deficits in synapse density of the CA1 and CA3 by 9 months for both general and glutamatergic synapses (Table 1a–b). There is further evidence that reduced glutamate metabolism in late stage AD pathology is specific to mitochondrial metabolism (Nilsen et al., 2014a), and more specifically mitochondria that reside in neuronal synapses (Volgyi et al., 2015). Therefore, GluCEST imaging appears to be most closely associated with the temporal and spatial trends in synaptophysin and GFAP immunostaining.

E5. Evidence of neuroprotective physiology

Increased glutamate levels in the dorsal hippocampus persisted through late stage pathology so that GluCEST levels in PS19 mice exceeded those in WT mice by 13 months, agreeing with our previous report (Crescenzi et al., 2014). Increased glutamate levels observed in the PS19 hippocampus may be consistent with evidence of neuroprotective physiology, specifically hyperperfusion and hypermetabolism. Evidence of regional hyperperfusion was presented in patients with FTL and AD using arterial spin-labeling (ASL) MRI (Hu et al., 2010). Another ASL-MRI study in a mouse model of tauopathy measured elevated cerebral blood flow (CBF) in the thalamus, cortex, and hippocampus (Wells et al., 2015). A caveat is that isoflurane increases CBF in the thalamus and cortex of mice, an effect that is resisted by the hippocampus (Zheng et al., 2009). It is possible that isofluorane elevates the absolute GluCEST signal given the involvement of glutamate neurotransmission in the regulation of blood flow. However, both studies appropriately control for these effects by imaging healthy mice that receive the same regimen of anesthesia.

While later stages of AD are characterized by reduced regional glucose metabolism using FDG-PET imaging (Landau et al., 2009; Jagust et al., 2010; Chen et al., 2011), reports of elevated glucose metabolism are measured in the hippocampus specifically (Dai et al., 2009; Alsop et al., 2008; Hu et al., 2010; Li et al., 2008). This is very interesting considering our findings of persistent elevation in GluCEST in the dorsal hippocampus of this tauopathy mouse model, while the ventral-caudal hippocampus is characterized by synapse loss and

decreased neuronal glutamate metabolism. It is reasonable that trends in glutamate metabolism would follow those of glucose metabolism, given that glutamate is a metabolic intermediate. Further, a state of elevated glucose metabolism and elevated glutamate metabolism potentially reflects a transient compensatory mechanism in response to synaptic failure. Whether GluCEST imaging as a measure of neuronal and glial glutamate metabolism would correlate with measures of gray matter perfusion or glucose metabolism remains to be investigated. This is an important opportunity for future studies to apply GluCEST imaging as a complimentary marker of brain metabolism, which may be useful as neurogenic therapies are developed (Chadwick et al., 2011; Chohan et al., 2011; Wang et al., 2010).

E6. Considerations for future studies in PS19 mice with tauopathy

Future studies in this mouse model should consider the time course of disease presented here, and the relevant regions of pathologic symptoms. The middle hippocampus slice (centered on Bregma -2.18 mm) was sufficient to show the greatest and earliest significance difference in GluCEST levels. Furthermore 9 months is a sufficient endpoint where pathologic events including the formation of hyperphosphorylated tau, synapse loss, and metabolic dynamics in hippocampal glutamate have already occurred prior to consistent volume loss. A practical limitation of this study is that the histology cohorts are not the same as those imaged, and tissue was obtained retrospectively at a slightly earlier age of 6 months. WT littermate tissue was not available at ages 6 and 9 months, therefore normal aging dynamics in VGlut1 for instance remain unknown in this cohort.

Regarding longitudinal timepoints, the interval between 7 to 9 months marks a shift in location of GluCEST deficits from the dorsal to ventral hippocampus. While dynamics in histopathology markers of synaptophysin, VGlut1, and GFAP were observed in this study at 3 month intervals, a relevant extension of this study would be to measure glutamate and IHC at finer intervals. Several remaining questions could be investigated: what are the intermediate histologic events between synapse loss and volume loss associated with GluCEST dynamics? Is there a breaking point of cellular stress which triggers neuronal death? For instance, calpain I is a calcium-dependent protease whose activity yields reactive oxygen species (McCord 1985). Calpain I was discovered to be a necessary mechanistic link between NMDA-excitotoxicity and pathologic tau degradation leading to cell death (Amadoro et al., 2006). Regarding measured elevations in GluCEST, if GluCEST in young PS19 mice is indeed a measure of excitotoxicity, how is glutamate metabolism affected before neuronal mitochondrial dysfunction? Subsequently, is the increase in dorsal hippocampal glutamate protective? GluCEST imaging could aid *in vivo* detection of early pathophysiology and the time course of neuronal death or therapeutic rescue.

Investigating questions such as these will require different experimental methods at the tissue level to be paired with GluCEST imaging in order to further validate its molecular basis. While we have presented quantitative imaging and IHC methods, the two measures are not causative. For the purpose of this study, our goal was to associate GluCEST signal changes with an indirect measure of glutamate neurotransmission and metabolism using methods that are translatable to clinical neuropathology, as was called for in the field by

Serrano-Pozo et al. 2011. GluCEST MRI remains the only technique able to measure glutamate physiology in an intact system, and is presented here in a mouse model of tauopathy with associated synapse loss and astrogliosis to show its relevance as an imaging biomarker of dementia.

F. Conclusion

In vivo imaging of hippocampal glutamate confirms a gradient in glutamate levels *in vivo* along the dorsal-ventral and rostral-caudal axes of the mouse hippocampus. This gradient becomes steeper and polarized as tauopathy mice age, compared to normal aging WT mice. GluCEST levels in the hippocampus are decreased in early stages of PS19 tauopathy compared to WT littermates, which is concurrent with synapse loss before structural volume loss. Further, we have presented evidence of GluCEST signal dynamics associated with altered markers of glutamate neurotransmission, both VGlut1 as a pre-synaptic marker and NR1 as a post-synaptic marker. Subsequent elevations in GluCEST occurred in the dorsal hippocampus and were associated with a concurrent proliferation of glial cells marked by GFAP. Together, these results support GluCEST MRI as a valuable preclinical biomarker of histopathologic events in tauopathy, specifically the combined trends in synapse density and glial cell proliferation, which can be applied *in vivo* and analyzed in subhippocampal regions.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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Figure 1. Image acquisition and segmentation pipeline

i. Anatomical T2-weighted magnetic resonance images (MRI) acquired from a live mouse brain. The example images shown here are from a 3 month old PS19 mouse. Slice positions were chosen relative to the lambda marker (yellow) identified on the sagittal scout image. The three slices (1mm thick each) span the hippocampus from rostral to caudal. GluCEST asymmetry maps represent glutamate levels in vivo with high spatial resolution (156 μm^2 inplane). Gray matter regions such as the thalamus have higher glutamate levels, compared to white matter regions like the corpus callosum where glutamate levels are low.

iii. Hippocampus proper segmented from the T2-weighted MRI and applied to GluCEST maps represents the hippocampus region of interest (ROI).

iv. Histogram of GluCEST values in an example slice of the hippocampus. The k-means clustering algorithm segments three “clusters” or subregions within the hippocampus based on high, medium, and low GluCEST values.

v. The three clusters are anatomically correlated to the dorsal (D, red), intermediate (I, green), and ventral (V, blue) hippocampus. The cluster mask displays the final subhippocampal ROIs.

vi. Three corresponding slices of the hippocampus from the Allen Brain Atlas (Johnson et. al., 2010).

Hippocampal sub-regions of interest are outlined: dentate gyrus (DG), cornu ammonis (CA1, and CA3), and the cerebral spinal fluid (CSF). Each region is colored to indicate their approximate cluster position.

vii. An approximate anatomical model of the subhippocampal ROIs which contribute to each cluster (dorsal, intermediate, ventral) in each slice (rostral, middle, caudal). In younger mice the CA1 extends through the caudal-ventral hippocampus, which later becomes CSF as mice age (arrow).

Figure 2. Example longitudinal GluCEST maps in multiple slices of the hippocampus
Representative GluCEST maps in the hippocampus are shown here from a PS19 mouse and a healthy WT mouse as they age from 3 to 13 months. At 9 months maps are shown from different mice due to the fact that only Cohort 2 was imaged at this timepoint. All three slices are from the same mice

Figure 3. Glutamate gradient in the hippocampus measured in vivo by GluCEST MRI
a–b) Group average GluCEST asymmetry (average CEST %) values from subhippocampal ROIs arranged on an anatomical grid. A gradient in glutamate levels is observed along the rostral-caudal and dorsal-ventral axes of the hippocampus. In WT mice there is a ubiquitous increase in GluCEST levels as mice age to 7 months; glutamate levels decline back to baseline by 13 months. PS19 mice have progressively decreased GluCEST throughout lifetime in most regions. An increase in GluCEST is measured among the dorsal regions of PS19 mice from 9 to 13 months. c) The differences in GluCEST asymmetry (WT values subtracted from PS19 values) were compared for each cluster using a Student's t-test. A (+) indicates higher GluCEST in PS19 mice compared to WT. Red highlight (>5 CEST % or significant difference $*p < 0.001$) is added to visually show the shift in GluCEST differences from the dorsal-caudal hippocampus in early stages of PS19 tauopathy, to the ventral-caudal hippocampus in later stages as tissue is lost.

Figure 4. Longitudinal volume changes in subregions of the hippocampus

The volume of each subhippocampal ROI normalized with the total volume of the hippocampus. Graphs are arranged according to their anatomical position (see Figure 1vii). Significant differences are measured in the more caudal-ventral regions by 13 months. Significance is reported between WT and PS19 group averages at each age using the Student's t-test. *p < 0.001.

Figure 5. Synaptophysin immunostaining and quantification of presynapse density

a) An example section through the hippocampus at approximately Bregma -2.05 mm, corresponding to the mid-slice which was imaged. The black box outlines the region shown in part (c). b) Regions of interest including the dentate gyrus (DG), CA1, and CA3 were segmented from 10x images, and percent area occupied (%AOC) was quantified. c) Representative immunostaining in the CA3 of PS19 and WT mice at various ages. d) Group average synaptophysin %AOC in the CA3 is significantly lower in PS19 mice at 6 and 9 months compared to the WT cohort at 3 months (* $p = 0.004$). The same trend occurs in the CA1 ($p = 0.004$) and DG ($p = 0.01$, see Table 1a).

Figure 6. GFAP immunostaining and quantification in the DG

a) GFAP contrast in a PS19 mouse at 9 months compared to a healthy-aging WT mouse at 13 months. b) Representative immunostaining in the DG of PS19 and WT mice at various ages. All images are shown at 20x magnification. c) Group average GFAP %AOC in the DG sub-region is significantly increased at 9 months in PS19 mice compared to WT at 3 months (* $p < 0.004$). Aged WT mice at 13 months also show significantly elevated GFAP levels (* $p < 0.004$), though to a lesser degree. A significant trend was measured in all subregions ($p < 0.004$, see Table 1c).

Table 1
Quantification of IHC for various antibodies in each sub-region of the hippocampus

Group average %AOC $\pm$ standard error of the mean are reported. Student's t-tests were performed for each region and time-point against the WT 3 month (mo) cohort. Significance after Bonferroni correction for comparisons of 3 regions and 4 time-points is ** $p < 0.004$, and for comparisons of 4 time-points for one region is * $p < 0.01$.

		WT				PS19			
		3 mo	13 mo	3 mo	6 mo	3 mo	6 mo	9 mo	6
a) Syn	#N	4	8	4	6	4	6	6	
	DG	69.9 $\pm$ 0.9	69.7 $\pm$ 1.5	67.5 $\pm$ 1.9	64.3 $\pm$ 1.5 *	64.3 $\pm$ 1.5 *	58.1 $\pm$ 3.3 *		
	CA1	65.5 $\pm$ 0.1	63.2 $\pm$ 2.9	63.0 $\pm$ 1.9	61.6 $\pm$ 0.8 **	61.6 $\pm$ 0.8 **	53.0 $\pm$ 2.6 **		
	CA3	70.6 $\pm$ 1.3	65.6 $\pm$ 1.7	67.3 $\pm$ 1.4	63.0 $\pm$ 1.1 **	63.0 $\pm$ 1.1 **	47.9 $\pm$ 2.7 **		
b) VGluT1	#N	4	6	4	5	4	5	6	
	DG	76.2 $\pm$ 1.4	71.2 $\pm$ 3.7	66.0 $\pm$ 3.1	77.9 $\pm$ 1.8 ‡	77.9 $\pm$ 1.8 ‡	64.9 $\pm$ 3.5		
	CA1	71.0 $\pm$ 1.6	66.8 $\pm$ 3.3	65.1 $\pm$ 6.6	74.4 $\pm$ 1.4	74.4 $\pm$ 1.4	62.7 $\pm$ 3.1		
	CA3	77.1 $\pm$ 0.8	70.9 $\pm$ 3.2	72.5 $\pm$ 4.4	78.5 $\pm$ 1.3	78.5 $\pm$ 1.3	57.1 $\pm$ 5.4 *		
c) GFAP	#N	4	7	4	5	4	5	6	
	DG	5.4 $\pm$ 1.1	25.5 $\pm$ 3.2 **	4.6 $\pm$ 1.2	4.3 $\pm$ 1.5	4.3 $\pm$ 1.5	79.5 $\pm$ 6.6 **		
	CA1	3.4 $\pm$ 0.7	20.5 $\pm$ 3.8 **	2.5 $\pm$ 0.3	3.3 $\pm$ 1.1	3.3 $\pm$ 1.1	61.2 $\pm$ 11.0 **		
	CA3	2.6 $\pm$ 0.8	14.0 $\pm$ 3.0 *	1.9 $\pm$ 0.3	2.4 $\pm$ 1.0	2.4 $\pm$ 1.0	64.7 $\pm$ 7.5 **		
d) NR1	#N	4	5	4	5	4	5	¥	
	DG	0.33 $\pm$ 0.16	0.59 $\pm$ 0.38	0.18 $\pm$ 0.09	0.11 $\pm$ 0.04	0.18 $\pm$ 0.09	0.11 $\pm$ 0.04		
	CA1	0.44 $\pm$ 0.16	0.45 $\pm$ 0.19	0.15 $\pm$ 0.08	0.08 $\pm$ 0.03	0.15 $\pm$ 0.08	0.08 $\pm$ 0.03		
	CA3	0.73 $\pm$ 0.33	0.76 $\pm$ 0.30	0.27 $\pm$ 0.11	0.15 $\pm$ 0.04	0.27 $\pm$ 0.11	0.15 $\pm$ 0.04		

#N: number of mice included in each group average.

¥ NR1 group average at 6 mo includes N=4 PS19 6 mo and N=1 PS19 9 mo

* p 0.01;

** p 0.004 compared with WT at 3 mo

$p=0.02$ compared with PS19 at 3 mo

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