Identification of changes in neuronal function as a consequence of aging and tauopathic neurodegeneration using a novel and sensitive magnetic resonance imaging approach

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Abstract

Tauopathies, the most common of which is Alzheimer's disease (AD), constitute the most crippling neurodegenerative threat to our aging population. Tauopathic patients have significant cognitive decline accompanied by irreversible and severe brain atrophy, and it is thought that neuronal dysfunction begins years before diagnosis. Our current understanding of tauopathies has yielded promising therapeutic interventions but have all failed in clinical trials. This is partly due to the inability to identify and intervene in an effective therapeutic window early in the disease process. A major challenge that contributes to the definition of an early therapeutic window is limited technologies. To address these challenges, we modified and adapted a manganese-enhanced magnetic resonance imaging (MEMRI) approach to provide sensitive and quantitative power to detect changes in broad neuronal function in aging mice. Considering that tau tangle burden correlates well with cognitive impairment in Alzheimer's patients, we performed our MEMRI approach in a time course of aging mice and an accelerated mouse model of tauopathy. We measured significant changes in broad neuronal function as a consequence of age and, in transgenic mice, before the deposition of *bona fide* tangles. This MEMRI approach represents the first diagnostic measure of neuronal dysfunction in mice. Successful translation of this technology in the clinic could serve as a sensitive diagnostic tool for the definition of effective therapeutic windows.

Keywords

MEMRI, tau, tangles, manganese, rTg4510, Alzheimer

Abbreviations

MRI: Magnetic resonance imaging

MEMRI: manganese-enhanced MRI

PET: positron emission tomography

AD: Alzheimer's disease

PSP: progressive supranuclear palsy

FTD: fronto-temporal dementia

CA1: cornu ammonis 1

CA3: cornu ammonis 3

DG: dentate gyrus

CTX: cortex

1. Introduction

Alzheimer's disease (AD) and related tauopathies are the most crippling cognitive threat to the aging population. There is no cure for tauopathies, and this is partly because of unclear understanding of tau pathogenesis. It is expected that in the United States we will spend approximately \$1.2 trillion to maintain the constantly deteriorating quality of life of 16 million Americans with AD by 2050 (Alzheimer's, 2016). Despite promising results in *in vivo* models, therapeutic interventions fail to halt the disease process, and they only provide temporary benefits. A major challenge in the field is the lack of sensitive technologies to identify pre-symptomatic signs of disease. Consequently, neuronal damage in the patient population recruited for clinical trials might be too far advanced for therapeutics to be effective. Therefore, there is a critical need to identify an early therapeutic window in the prodromal stage of disease.

The current diagnostic imaging measure of pre-AD relies on amyloid plaque detection. Individuals are screened using anti-amyloid ligands (florbetapir) with positron emission tomography (PET) (Carome and Wolfe, 2011; Choi et al., 2009; Klunk et al., 2004; Mathis et al., 2004). In addition, development of effective and specific tau tangle ligands such as ^{18}F -T807 accelerated the possibility of measuring both pathological signs of AD and discriminating several diseases (Chien et al., 2014; Johnson et al., 2016; Marquie et al., 2015). There are several challenges for using PET technology to diagnose neurodegenerative processes. Accessibility to PET instruments is limited by location and cost, curtailing widespread availability of this technique. Moreover, the merging of PET molecular signatures to anatomical features requires the addition of structural imaging and in the case of computed tomography imaging, which leads to

double exposure to radiation. The most pivotal obstacle with current imaging diagnostics is their focus on correlation between cognitive damage and mature pathology. Individuals in the prodromal stages of disease are only identified after PET-positive amyloid pathology appears. Moreover, [F18]-AV-1451 tracers detect PHF1-positive aggregates, which correspond to late stage tangles enriched with pS396/S404 tau. Therefore, individuals who present pathological signs of disease and mild (mild cognitive impairment) to moderate symptomatology likely suffer simultaneous irreversible neurodegenerative processes making therapeutic interventions less efficacious (Dubois et al., 2016).

In contrast, access to MRI technology is widely available, and does not require the use of ionizing radiation. MRI can be combined with contrast enhancement agents to increase the sensitivity of this technique. Manganese-enhanced MRI (MEMRI) offers a powerful tool to repeatedly measure neuronal function in longitudinal *in vivo* studies. MEMRI is particularly well suited for brain imaging because manganese enters voltage-gated ion channels proportionally to calcium flux (Lin and Koretsky, 1997) and shortens the T1 relaxation time of neighboring water (Antkowiak et al., 2012; Bissig and Berkowitz, 2014; Drapeau and Nachshen, 1984; Koretsky and Silva, 2004; Vandsburger et al., 2012). Once in a neuron, manganese is trafficked through the cells and released in the post-synaptic density (Pautler et al., 1998), and this activity-dependent uptake and trafficking correlates with broad neuronal function (Pautler and Koretsky, 2002). In addition, manganese is taken up preferentially in the hippocampus (Shineman et al., 2011), a key brain region involved in learning and memory. Therefore, MEMRI is a powerful technique to quantify broad neuronal function in brain regions affected by age and neurodegenerative disease.

We tested the value of combined MEMRI and mapping of T1 relaxation times coupled with T2-mapping to determine changes in signatures over time and with disease in the rTg4510 transgenic mouse model of accelerated tauopathy (Santacruz et al., 2005). MEMRI recognized alterations in broad neuronal function during in pre-symptomatic stages of pre-tangle pathology. Our results suggest that MEMRI is a sensitive, widely available, and non-invasive *in vivo* imaging technique for identification of early stages of neurodegeneration.

2. Materials and Methods

2.1 Mice and Ethics Approval

All animal studies were approved by the University of Kentucky's Institutional Animal Care and Use Committee (IACUC) and abided by that committee's policies on animal care and use in accordance with the Guide for the Care and Use of Laboratory Animals, the Animal Welfare Regulations Title 9 Code of Federal Regulations Subchapter A, "Animal Welfare," Parts 1–3, and the Public Health Service Policy on Humane Care and Use of Laboratory Animals. This University of Kentucky program and the facilities for animal care and use are fully accredited by the Association for Assessment and Accreditation of Laboratory Animal Care International. The animals were kept in standard housing on a 14h light /10h dark cycle and received food and water *ad libitum*. The tau transgenic (rTg4510) and parental mice (rTta and TRE-4R0N-P301L MAPT, on a FVB background) were maintained and genotyped as described previously (Santacruz et al., 2005). All experiments were done in both male and female mice.

For MEMRI studies, at each age group (2, 3, and 10 months) a naïve group of mice were subjected to one MEMRI scan. As we observed no differences in MEMRI between TRE-4R0N P301L MAPT (tau), rTta and wt littermates, each experiment was performed with age and sex matched littermates of the non-rTg4510 genotype (ctrl).

2.2 Manganese-enhanced magnetic resonance imaging (MEMRI)

All MRI procedures were performed in accordance with the University of Kentucky Magnetic Resonance Imaging and Spectroscopy Core (MRISC) IACUC Protocol. Manganese, which is identical in charge and similar in ionic radius to calcium, enters voltage-gated calcium channels on neuronal membranes, follows calcium trafficking, and is released in the synaptic cleft and internalized by the postsynaptic neuron (Masumiya et al., 2003; Pautler et al., 1998). Manganese is also a potent MRI contrast agent that lengthens R1 ($1/T_1$) relaxation rates as a function of concentration in tissue (Vandsburger et al., 2012). When MEMRI is combined with an imaging protocol to quantify tissue R1 relaxation rates, the acquired data reflects an *in vivo* measurement of underlying function of cells with voltage gated calcium channels (Antkowiak et al., 2012). Manganese chloride (30mM) prepared in saline was delivered to mice via intraperitoneal injection (66mg/kg). All MR imaging was performed on a horizontal bore 7T Bruker ClinScan magnet (Bruker, Ettlingen, Germany) using a cylindrical volume coil for excitation and a cryocoil for detection. In preparation for imaging, animals are anesthetized using isoflurane (3-5%) in oxygen at a rate of 0.5-1.0 L/min and maintained using 1-3% isoflurane in oxygen. Body temperature was maintained using circulating water, and vital signs (core temperature and respirations) were monitored using a physiological monitoring system SA Instruments Inc. (SAI, Stony Brook, New York). Look-

Locker imaging was performed following non-selective spin inversion in one slice of the brain containing large regions of hippocampus. Fifty images were acquired following inversion with image spacing of 100ms (total sequence repetition time of 5s) to fully sample the T1-relaxation curve. Additional image parameters included TR/TE = 5500/1.9, Matrix = 128 x 128, number of averages = 3, field of view = 17mm x 17mm x 0.7mm. T2-weighted images were acquired covering the entire brain (excluding the cerebellum and olfactory bulb) using a turbo-spin echo sequence with TR/TE = 3360/42, Slices = 21, Matrix = 448 x 336, number of averages = 2, field of view = 25mm x 25mm x 0.5mm. The imaging procedures for scanning one mouse were completed in 45min. Imaging was performed prior to the injection of MnCl₂ (baseline) and repeated at 6h post-injection (Fig. 1). Image mapping and analysis was performed in MATLAB (Mathworks, Natick, MA, USA). Images from the Look-Locker series were used to reconstruct voxel-by-voxel signal relaxation curves which were fit to the equation $S(TI) = S_0 \cdot (1 - e^{-R_1 \cdot TI})$, where S(t) represents the signal at a given inversion time (TI), S₀ represents the steady state signal at maximal TI, and R₁ represents the longitudinal relaxation rate. Regions of interest (dentate gyrus, CA1, CA3, and superior medial cortex) were identified using the Allen Brain Mouse Atlas. Within each the change in R₁ relaxation rates (ΔR_1) before and after MnCl₂ exposure was calculated as

$$\Delta R_1 = R_{1_{6h}} - R_{1_{baseline}}$$

2.3 Immunohistochemistry

Following MEMRI, mice were irreversibly anesthetized, exsanguinated, and transcardially perfused with saline followed by 4% phosphate-buffered paraformaldehyde

(PFA, pH 7.4), a protocol mildly modified from (Abisambra et al., 2010b). Brain samples were fixed in 4% paraformaldehyde for 24 hr, then cryoprotected by incubating in successive 24 h increments with 10%, 20%, and 30% sucrose gradients as described previously (Jinwal et al., 2010). Brains were frozen on a temperature-controlled freezing stage, sectioned (25 μ m) on a sliding microtome, and stored in a solution of PBS containing 0.02% sodium azide at 4°C.

Free-floating immunohistochemistry was performed as described (Abisambra et al., 2010a). The following antibody dilutions were used: pS262 (Anaspec) 1:10,000; pT231 (Invitrogen) 1:25,000; MC1 (kind gift from P. Davies) 1:2500. IHC sections were imaged using a Zeiss AxioScan (Zeiss, Germany). Image quantification was performed using ImageJ as follows: regions of interest (CA1, CA3, DG, and cortex) in 2-4 coronal sections per mouse were quantified. Then, all images were thresholded identically and intensity was measured for each region of interest. Quantifications shown are mean $\pm$ standard error of the mean.

2.4 Statistical analysis

All statistical analyses for MEMRI were performed using GraphPad Prism 6 (Graph Pad Software, Inc. La Jolla, CA, USA). Results are shown as the mean $\pm$ standard error. Data from figure 2 were analyzed using two-way ANOVA with a Bonferroni posttest. Data from figures 3 and 4 were analyzed using a student's unpaired t-test. A $p < 0.05$ was considered significant. For immunohistochemistry, any outliers two standard deviations from the mean were excluded. Two way ANOVAs with multiple comparison

post-hoc tests were performed to identify statistically significant differences using GraphPad Prism 6 software. A confidence interval of 95% ($p < 0.05$) was considered statistically significant.

3. Results

We first investigated MEMRI viability as a method to reveal age-related changes in wild type mice. Since aging impacts calcium homeostasis, overall learning and memory, and neuronal connectivity (Gray and Johnston, 1987; Landfield, 1988; Sastry et al., 1986), we speculated that MEMRI could detect age-dependent alterations in brain regions where these functions are crucial. We first established a baseline model in control mice by performing MEMRI scans at in groups of mice aged two, three, six, and ten months of age. Representative coronal sections of high-resolution R1 maps are shown for each age group (Figure 2A-D). Quantification of $\Delta R1$ revealed increased changes in relaxation rates ($\Delta R1$, where $R1 = 1/T1$) in hippocampal regions CA1, CA3, and DG (Figure 1A, Figure 2E-G) as well as in the superior medial cortex (Figure 2H). In all regions, there was an initial increase in $\Delta R1$ values from two to three months, followed by a sharp decrease in $\Delta R1$ values as animal age increases (Figure 2E-H), suggesting manganese uptake decreases with age. These results suggest MEMRI is a sensitive and quantitative technique to distinguish age-related alterations in non-transgenic mice. We next sought to determine whether $\Delta R1$ values could distinguish differences between age-related and neurodegenerative disease-associated alterations.

MEMRI successfully detected differences in AD mouse models overexpressing APP (Smith et al., 2007) and has been used to examine axonal transport rates in olfac-

tory bulbs of rTg4510 (Majid et al., 2014). Because cognitive decline is closely associated with pathogenic tau accumulation, we used rTg4510 mice to identify whether MEMRI specifically detects changes in regions of the brain negatively impacted by tauopathy. Tau transgenic rTg4510 mice express very high levels of human tau with a pathogenic mutation associated with FTDL17 (Santacruz et al., 2005). Pre-tangle pathology matures into *bona fide* Alzheimer's-like tau tangles by four months of age (Ramsden et al., 2005; Santacruz et al., 2005). This pathological transition precedes moderate yet significant electrophysiological deficits and cognitive impairments, which become evident after 3.5 months and 4 months, respectively (Abisambra et al., 2013; Abisambra et al., 2010b; Ramsden et al., 2005). By 5.5 months, rTg4510 mice show overt tangle pathology along with significant cognitive decline and brain atrophy (Santacruz et al., 2005). These signs and symptoms are further potentiated with age: at ten months, rTg4510 mice show larger tangles reminiscent of those present in fronto-temporal lobar degeneration brains, ~20% reduction in brain weight, and more severe cognitive deficits than 5.5-month-old transgenic mice (Ramsden et al., 2005).

We hypothesized that alterations in broad neuronal function caused by severe tau pathology would be detectable by MEMRI. We performed MEMRI $\Delta R1$ analyses in 10mo rTg4510 transgenic mice, an advanced stage in the disease showing significant brain atrophy, tau pathology, and cognitive impairment. Indeed, MEMRI $\Delta R1$ revealed gross abnormalities in rTg4510 brain morphology: cortical thinning (Figure 3B) and accumulation of cerebrospinal fluid (blue color, Figure 3B). Comparison of pre- and post-contrast R1 maps revealed that rTg4510 mice have significantly increased $\Delta R1$ (Figure 3B-C) in CA1, and dentate gyrus regions of the hippocampus, as well as in the cortex

compared to age-matched littermate control mice (Figure 3A-C). There was no difference in any MEMRI signatures in the rTg4510 littermate controls (MAPT, tTA, or wt). These data suggest that rTg4510 mice have a much greater rate of manganese uptake and retention in the brain. Interestingly, we observed a significant decrease in $\Delta R1$ from 3mo to 10mo of age (Figure 3I). The only significant decrease between the different regions over time occurred in CA3.

We then tested the sensitivity of MEMRI to identify alterations in rTg4510 mice prior to the onset of cognitive decline. While deficits in synaptic plasticity are detected as early as 3.5 months in rTg4510 (Abisambra et al., 2013), robust deficits in cognition are readily detected in these animals at 4 months of age (Ramsden et al., 2005). We examined mice at 2 and 3 months of age, prior to the onset of severe cognitive damage. At two months, $\Delta R1$ was unchanged between rTg4510 mice and littermate controls (Fig. 4). At three months, rTg4510 showed significant differences in $\Delta R1$ in the CA1, CA3, dentate gyrus, and cortical brain regions compared to 2-month-old rTg4510 mice (Fig. 4). Compared to littermate controls, $R1$ in the CA1 and CA3 region of the hippocampus was significantly increased (Fig. 4). Thus, specific tau-related changes in neuronal dysfunction are detectable by MEMRI as early as 3 months of age, prior to the onset of severe cognitive impairment in a transgenic tau animal model. These data suggest that MEMRI can detect changes in broad neuronal function prior to cognitive impairment.

To identify how closely MEMRI deficits are associated with increased pathogenic tau, we performed immunohistochemistry on 2- and 3-month-old rTg4510 mice. We first measured an early pathological tau conformation detected by the antibody MC1 (Weaver et al., 2000). As expected, MC1 immunoreactivity was present in hippocampal

sub-regions and the cortex of both 2- and 3-month-old rTg4510 mice (Fig. 5). However, MC1 immunoreactivity was significantly increased in the CA3, dentate gyrus, and the superior medial cortex regions of these three-month-old tau transgenic mice (Fig. 5).

We next measured the presence of phosphorylated tau species present in pre-tangle pathology. Tau is phosphorylated at Ser262 and T231 early during in disease progression in AD and other diseases (Goedert et al., 1995; Hirano et al., 1968). Despite the appearance of pS262 and pT231 in pre-tangle pathology (Augustinack et al., 2002), we did not detect differences in the signal for these phospho-tau species between 2- and 3-month-old rTg4510 mice (Supplemental Fig. 1). Thus, these data suggest that pre-symptomatic detection of tau-related changes in neuronal function by MEMRI are associated with the accumulation of the earliest conformational markers of pathogenic tau, but not tau hyperphosphorylated at pS262 or pT231.

4. Discussion

We used MEMRI coupled with high resolution R1 mapping to measure broad neuronal function in mice. We identified longitudinal changes in small functional hippocampal regions (Fig. 2), significant neuronal changes in aged tau transgenic mice (Fig. 3), and the earliest sign of neuronal dysfunction in tauopathic hippocampus and cortex (Fig. 4). These latter changes coincide with appearance of MC1-positive tau but not phospho-tau protein aggregates (Fig. 5). An important and critical finding of these studies is the identification of the earliest sign of brain alterations in a well-characterized tau mouse model, suggesting that MEMRI-R1 can be used to identify the beginning of a

viable and potentially more effective therapeutic window in brains with neurodegeneration.

This is a highly sensitive approach to perform sub-regional analyses, which permits quantification of broad neuronal function in small areas of interest. The derived data offer unique insights into tauopathies by establishing areas most susceptible to tau-mediated neuronal damage. Future translational efforts could also use MEMRI-R1 to identify subtle changes in neuronal function in small regions of the brain in response to interventions.

The molecular mechanisms governing manganese uptake, retention, and clearance in brain cells remain incompletely understood. In ten-month-old transgenic mice, we detected significantly increased $\Delta R1$ in all the regions analyzed except for the CA3 (Fig. 3). Similarly, $\Delta R1$ of three-month-old transgenic mice was significantly increased from age-matched non-transgenic controls in CA3 and CA1 but not in DG or CTX (Fig. 4). The presence of elevated changes in R1 values, correlating with heightened cellular levels of manganese, suggests that normal pathways for calcium homeostasis are impacted. Here, it is possible that MEMRI- $\Delta R1$ detects calcium dysregulation, which is a major sign of neuronal dysfunction that has been previously described (Decker et al., 2015). These data also suggest that the mechanisms mediating calcium dysregulation in tauopathy also regulates evident manganese mis-sorting. Therefore, manganese is an effective calcium surrogate with tremendous potential to reveal changes in calcium dynamics, as an early sign of dysfunction in disease. In addition, using manganese to monitor calcium pathways would be a powerful means to measure quantitatively the effect of treatments on neuronal function. Data from Figure 3F demonstrates that $\Delta R1$ in

CA3 decreases significantly over time. Together with the suggestion that CA3 plays an important role in disease progression of rTg4510 mice, our data suggest that the CA3 undergoes significant and early changes in rTg4510, which do not persist through 10mo compared to controls. Despite lack of persistence, these changes suggest that CA3 certainly plays a distinct and crucial role in disease progression worthy of further investigation.

Despite the $\Delta R1$ aberrancies in tau transgenic mice, our approach revealed distinct $\Delta R1$ patterns at three months of age in all imaged brains. In non-transgenic mice, $\Delta R1$ values increased at three months (Fig. 2). This pattern was significantly increased in three-month-old rTg4510 mice compared to two month-old rTg4510, and both two- and three month- old littermate controls (Fig. 4). These data highlight important neurological processes at three months, which are altered in rTg4510 mice before the appearance of significant tau pathology and cognitive impairment.

Our approach builds on previous studies (Antkowiak et al., 2012; Lin and Koretsky, 1997; Pautler, 2004; Pautler et al., 1998; Vandsburger et al., 2012) to measure changes in the brain. MEMRI offers a powerful approach to quantitatively perform functional and minimally invasive measurements in the intact brain. Alternatives that reveal functional measurements, particularly in early stages of tauopathy, are terminal procedures such as electrophysiological measurements (Abisambra et al., 2013). Other studies employing MEMRI to measure changes in rTg4510 mice have also shown important differences. In one instance, MEMRI was used to measure transport rates and tract tracing in the olfactory bulb and showed that rTg4510 mice present transport deficits along olfactory axons (Majid et al., 2014). These data in the olfactory tract suggest

and support our findings that there are neuronal deficits in the hippocampus of tau transgenic mice.

Another study by Perez and colleagues observed neuronal dysfunction in the CA3 of five-month-old rTg4510 mice using MEMRI (Perez et al., 2013). Since tau pathology ensues well before five months in rTg4510 mice, it was reasonable to measure changes at earlier time points, which led us to identify MEMRI deficits as early as three months of age in rTg4510 mice (Fig. 4). Our approach expands on this finding by adding sensitivity to detect abnormal patterns of neuronal dysfunction in the transgenic mice in specific sub-regions (DG, CA1, CA3, and CTX) as well as significant differences in both the CA1 and CA3 when compared to non-transgenic mice. The ability to detect differences in neighboring sub-regions of the hippocampus suggests that MEMRI is highly sensitive. These data also foreshadow a mechanism of disease showing that CA1 and CA3 are the first regions to be affected by tau overexpression in the forebrain. Our results are supported by previous findings showing that rTg4510 mice present significant tau deposition in CA1 of two-month-old mice, and that tau aggregation spreads to CA3; tau pathology finally reaches the DG by eight months (Ramsden et al., 2005).

Despite the robust differences measured in CA1, DG, and CTX of ten-month-old rTg4510 mice, we were surprised to find that the CA3 did not show significant differences from controls. One possible explanation is that $\Delta R1$ in CA3 is suppressed due to more neuronal atrophy in this region compared to other sub-regions of the hippocampus. This would lead to a reduction of voltage gated calcium channels that would impair manganese influx (Pautler, 2011; Lee and Koretsky, 2005). Interestingly, neuronal dys-

function is detected in rTg4510 mice at three months in the CA3 and CA1 regions of the hippocampus when compared to non-transgenic mice (Fig. 4).

An important consideration in this study is that the rTg4510 model is characterized by aggressive pathology (Ramsden et al., 2005; Santacruz et al., 2005). This is in part due to increased expression (approximately thirteen times more) of human P301L tau, which is associated with onset of fronto-temporal dementia. It would certainly be interesting to establish the effect of wild type tau overexpression on $\Delta R1$ changes. Since overexpression of wild type tau leads to tauopathic aberrancies (Duff et al., 2000), and many other tauopathies are characterized by non-P301L tau tangles, we would expect to determine $\Delta R1$ changes; however, the extent of these changes is unknown. On the other hand, the mechanisms of cell damage imparted by P301L and wild type pathological tau could be different, and as a result $\Delta R1$ might be normal in the latter condition. If this is the case, our approach would be more pertinent to FTD, and as such, it would be a unique approach to differentiate FTD from other non-P301L tauopathies AD.

Electrophysiological measurements show impaired long-term potentiation deficits as early as 3-3.5 months in rTg4510 mice suggesting circuitry malfunction in the CA1-CA3 (Abisambra et al., 2013). Other studies have indicated synaptic density deficits as well as spine loss in the rTg4510 mice at 7-9 months without calcium dysregulation (Kopeikina et al., 2013; Kuchibhotla et al., 2014). Therefore, compensatory mechanisms could be occurring in the circuitry at later time points to adapt to the changes occurring in CA3 at the early time point of 3mo where neuronal dysfunction is first detected in the hippocampus (Fig. 4). Electrophysiological approaches to measure calcium dysregulation in rTg4510 mice at three months of age are currently underway and could highlight

a major molecular mechanism leading to neuronal dysfunction in early stages of the disease.

5. Conclusions

This study supports the use of MEMRI and R1 mapping as a sensitive and quantitative technique to identify changes in broad neuronal function as a consequence of development, aging, and disease. Moreover, the MEMRI application described herein has the potential to reveal early therapeutic windows for disease and monitor the impact of treatments in patients. A major step forward in the field will be translational efforts to use manganese-based FDA approved compounds in humans such as mangafodipir. Finally, these data demonstrate that neurological dysfunction coincides with appearance of MC1 tau and before deposition of hyperphosphorylated tau pathology.

Declarations

All authors consent publication of this manuscript. There are no competing interests. Funding for this work came from the University of Kentucky Alzheimer's Disease Center (UK-ADC), which is supported by NIH/NIA P30 AG028383. JFA, SNF, AI, RAC. SEM, EM, DL, GKN, and EM, were supported by NIH/NINDS 1R01 NS091329-01, Alzheimer's Association NIRG-14-322441, NIH/NCATS 5UL1TR000117-04, NIH/NIGMS 5P30GM110787, Department of Defense AZ140097, the University of Kentucky Epilepsy Center (EpiC), NIH/NIA P30 AG028383, and NIH/NIMHD L32 MD009205-01. We ac-

knowledge Dr. Donna Wilcock for use of the AxioScan, Dr. Peter Davies for the MC1 antibody, and Dr. Frederick Schmitt for comments on this manuscript and insights into these experiments. Author contributions are as follows: JFA designed the study. SNF, AI, SEM, EM, and DL performed MRI. MV developed analysis program for MEMRI and MV, DL, SEM, and RAC analyzed MEMRI data. AI and EM performed mouse husbandry and with SNF, tissue collection. SNF performed all IHC and analysis. SNF, MV and JFA interpreted data and wrote the paper. Data and materials will be made widely available upon reasonable request to the corresponding author.

Figure legends:

Figure 1. Pre and post-manganese parametric $\Delta R1$ maps of a non-transgenic mouse. A) High resolution R1 mapping from baseline scans; regions of interest are denoted. B) Same mouse, R1 maps from scan taken 6h post-manganese injection.

Figure 2. MEMRI detects age-related changes in manganese uptake in non-transgenic mice. (A-D) Representative MEMRI R1 map of littermate control mice at A) 2mo, B) 3mo, C) 6mo, and D) 10mo. Quantification of $\Delta R1$ values in E) CA3 F) CA1 G) dentate gyrus (DG) and H) superior medial cortex (CTX). (I-J) $\Delta R1$ and one-way ANOVA analysis using Tukey's multiple comparisons test of different brain regions (DG, CA1, CA3, and CTX) in (I) 2mo, (J) 3mo, (K) 6mo, and (L) 10mo control mice. All values are mean $\pm$ SEM, n= at least 6 per group.

Figure 3. Significant MEMRI changes in aged tauopathic mice. Representative R1 maps of MEMRI-scans of 10mo littermate control (A) and 10mo rTg4510 (Tg) mice (B).

C) Quantification of $\Delta R1$ values in CA3, CA1, dentate gyrus (DG), and superior medial cortex (CTX). All values are mean $\pm$ SEM, $n=6$, $*p<0.05$.

Figure 4. MEMRI-R1 detects early signs of neuronal dysfunction in transgenic rTg4510 mice prior to the onset of cognitive deficits. (A-D) Representative R1 map of A) 2mo littermate control B) 3mo littermate control C) 2mo rTg4510 mouse (Tg) and D) 3mo rTg4510 mouse (Tg). Quantification of $\Delta R1$ values in E) CA1 F) CA3 G) dentate gyrus (DG), and H) superior medial cortex (CTX). (I) $\Delta R1$ comparison between different brain regions (CA3, CA1, DG, and CTX) in 3mo and 10mo rTg4510. All values are mean $\pm$ SEM, $n=$ at least 4. $***p<0.001$, $**p<0.01$ $*p<0.05$.

Figure 5. MEMRI coincides with increased signal of the earliest marker of tau pathology. (A-H) Representative immunohistochemistry micrographs (20x objective) from 2- and 3mo rTg4510 stained with MC1 tau. (I) Quantification of MC1 levels in CA1, CA3, DG, and CTX. Data are mean $\pm$ SEM, $n=3$ mice per group and 2-4 slices per mouse, $*p<0.05$. (J-L) Representative immunohistochemistry micrographs of 3mo rTg4510 mice in the regions denoted (CA3, DG, CTX) that were statistically significantly different and show tangle-bearing neurons and filaments (60x objective).

Supplemental Figure 1. MEMRI detects neuronal dysfunction prior to increased hyperphosphorylated tau. (A-H) Representative immunohistochemistry micrographs (20x objective) from 2- and 3mo rTg4510 stained with pS262 tau. Data are representative of $n=3$ mice per group and 2-4 slices per mouse. (I-P) Representative immunohistochemistry micrographs (20x objective) from 2- and 3mo rTg4510 stained with pT231 tau. Data are representative of $n=3$ mice per group and 2-4 slices per mouse.

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Figure 1.

Figure 2.

Figure 3.

Figure 4.

Figure 5.