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ORIGINAL ARTICLE

In vivo proton magnetic resonance spectroscopy detection of metabolite abnormalities in aged Tat-transgenic mouse brain

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Abstract Most individuals living with HIV in the USA are over 45 years old and are vulnerable to the combined effects of HIV and aging. Antiretroviral therapies reduce HIV morbidity and mortality but do not prevent HIV trans-activator of transcription (Tat) protein expression or development of HIV-associated neurocognitive disorder (HAND), which may be caused by Tat. Tat-transgenic (Tat-tg) mice are used to study Tat's effects, typically after transgene induction with doxycycline. However, uninduced Tat-tg mice experience transgene leak and model aspects of HAND when aged, including neuroinflammation. We used in vivo 9.4-tesla proton magnetic resonance spectroscopy to compare neurochemistry in aged versus young female and male uninduced Tat-tg mice. Aged Tat-tg mice demonstrated measurable *tat* mRNA brain expression and had lower medial prefrontal cortex (MPFC) GABA, glutamate, and taurine levels and lower striatal GABA and taurine levels. Females had lower MPFC glutathione and taurine and lower striatal taurine levels. Brain testosterone levels were negatively correlated with age in aged males but not females. Aged mice had cortical abnormalities not previously reported in aged wild-type mice including lower MPFC GABA and taurine levels. As glutathione and taurine levels reflect inflammation and oxidative stress, our data suggest that Tat may exacerbate these processes in aged Tat-tg mice. However, additional studies in controls not expressing Tat are needed to confirm this point and to deconvolve individual effects of age and Tat expression. Sex steroid hormone supplements, which counter climacteric effects, increase taurine levels, and reduce inflammation and oxidative stress, could attenuate some of the brain abnormalities we identified in aged Tat-tg mice.

Keywords Brain · Estradiol · Glutathione · HIV · Inflammation · Magnetic resonance spectroscopy · Oxidative stress · Sex difference · Tat · Taurine · Testosterone

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Introduction

In 2018, nearly 25% of new HIV cases in the USA occurred in people over 45 years old, and of those currently infected, more than half are older than 50 [1]. Thus, most people in the USA living with HIV are older and potentially vulnerable to the concurrent effects of HIV and aging [2]. The use of combined antiretroviral therapy (cART) substantially reduces morbidity and mortality associated with HIV but cART does not prevent the development of HIV-associated neurocognitive

disorder (HAND), which is highly prevalent and is associated with mood and cognitive impairments that reduce quality of life [3]. Reservoirs of HIV endure in the brain despite cART and continue to produce and release soluble HIV proteins, even when viral replication is nearly eliminated [3]. One such protein, transactivator of transcription (Tat), greatly potentiates HIV replication and may be critical in the reactivation of latent HIV reservoirs [4] as Tat protein expression persists and can even increase within the brain during cART [5–7].

The importance of Tat in HIV pathophysiology is demonstrable given that its infusion into animals or its expression in Tat-transgenic (Tat-tg) mice leads to the development of mood and cognitive impairments that recapitulate aspects of HAND [8–17]. Tat protein is suspect because it induces excitotoxicity [18], oxidative stress [14, 19, 20], inflammation [6, 8, 14, 21–25], and mitochondrial dysfunction [19, 26–28], all of which are associated with HAND.

Little is known about the effects of Tat protein in the aged brain, but recent studies in aged Tat-tg mice indicate that Tat accelerates age-related hormonal, physiological, neural, and cognitive decline [15, 17, 24], effects similar to those reported in older individuals living with HIV [29–32]. Prolonged Tat transgene "leak" in Tat-tg mice leads to the accumulation of Tat protein levels that are sufficient to induce brain inflammation, synaptic and axonal abnormalities, and ventricular volume increases over the course of a year [24]. Accordingly, Tat transgene leak in Tat-tg mice may model some aspects of Tat expression occurring in HIV patients on cART and thus merits study. Further, because Tat's deleterious effects may be enhanced by the presence of other HIV viral proteins [33, 34] or by cART medications [34–37], it is important to selectively study the effects of Tat in animal models unconfounded by other HIV proteins or cART medications, as part of a comprehensive strategy to better understand and treat HIV.

Presently, we used ultra-high-field (9.4 Tesla) in vivo proton magnetic resonance spectroscopy (MRS) to evaluate the effects of aging on cerebral metabolites in Tat-tg mice. In vivo MRS is noninvasive and thus enables longitudinal studies in animals, including in mice that are genetically similar to Tat-tg mice, to assess effects of aging [38]. MRS also facilitates within-subject longitudinal studies of the effects of potential interventions designed to target Tat or mitigate its effects. We previously used in vivo MRS to detect frontal cortex metabolite abnormalities indicative of oxidative stress in young adult male Tat-tg mice induced to express Tat protein for 7 days via doxycycline (Dox) administration [14]. In the present study, we used similar in vivo MRS methods in uninduced Tat-tg mice to examine the effects of aging and sex in frontal cortex and striatum, brain areas known to be affected by in vivo Tat exposure [14, 17, 20, 23, 39–45]. The Tat-tg mice we used contain an astrocyte-specific glial fibrillary acidic protein (GFAP) Dox-inducible promotor that controls expression of HIV-$Tat_{1\text{-}86}$ protein [46, 47]. However, in the absence of Dox administration, another strain of inducible Tat transgenic mice, the rtTA strain, is exposed to Tat via Tat transgene leak and Tat mRNA and protein levels accumulate at high levels sufficient to promote the development of brain structural abnormalities as well as inflammation [24]. We hypothesized that we would detect MRS metabolite abnormalities in aged uninduced Tat-tg mice indicative of oxidative stress and inflammation.

Methods

Subjects

Male and female GFAP/Tat-tg bigenic "Tat-tg" mice backcrossed at least 7 generations to a C57BL/6J mouse line [46, 47] were bred and maintained in the Animal Care Facility at McLean Hospital. After weaning, mice were segregated by sex and were group-housed (3–4/cage). Mice were maintained on a 12:12-h light/dark cycle and were provided with ad libitum access to food and water. All experimental procedures were reviewed and approved by the McLean Hospital Institutional Animal Care and Use Committee and were carried out in accordance with the National Institutes of Health Guide for the Care and Use of Laboratory Animals (NIH Publication Number 85-23). Adult mice aged 13–14 and 63–89 weeks (Table 1) were scanned once each in this cross-sectional cohort study. One female mouse (63 weeks old at the time of scanning) had been a breeder and had produced 4 litters). While breeding could have influenced her endocrine status [48], brain estradiol levels for this mouse were below the level of detection. Mice were not administered Dox or any other treatment in this study, which aimed to evaluate effects of aging and sex on MRS metabolites.

Magnetic resonance imaging and magnetic resonance spectroscopy

Anesthesia

Mice were anesthetized with 1–2% isoflurane and their vital signs, including rectal temperatures, heart-rates, respiration rates, and ECG, were continuously monitored (Small Animal Instruments, Inc., Stony Brook, NY). Body temperature and respiration rates were maintained by adjusting isoflurane levels as well as circulating water bath heating pad and warm air blower temperatures.

Imaging

Magnetic resonance imaging and spectroscopy (MRI/MRS) scans were acquired with a 9.4 tesla (T) horizontal bore system equipped with a Varian VNMRJ direct-drive console (Agilent, Santa Clara, CA), using a 12-cm (inner diameter) gradient. A custom designed and built (Life Services, LLC, Minneapolis, MN) quadrature butterfly surface coil was placed over the head. Turbo fast low-angle shot (TurboFlash) scout MRI images were acquired to position medial prefrontal cortex (MPFC) and left striatal voxels (Fig. 1, inset). MPFC and striatal MRS voxel shimming and water suppression were performed using the FASTMAP and VAPOR approaches, respectively [49, 50]. Localized proton (^{1}H) spectra (MPFC: 1.2 × 2.5 × 2.5 mm=7.5 mm^3; left striatum: 2.0 × 2.0 × 1.6 mm=6.4mm^3) were acquired with the LASER spectroscopy sequence [51] using a repetition time (TR)/echo time (TE) = 3000/19 ms. Bins of free induction decays (FIDs, every 8 scans) were separately acquired and stored automatically by the console and then corrected for frequency and phase shifts due to physiological or hardware variations. In total, 480 scans were acquired (24 min) for each voxel. Additionally, water-unsuppressed spectra (8 FIDS) were obtained from each voxel using identical parameters (but without VAPOR suppression), enabling metabolite concentration quantification by referencing to voxel water signals. Total scan times in each mouse were ≤ 2 h.

Table 1 Study subject demographics

Age group	♀ (mean, weeks ± SD; *N*)	♂ (mean, weeks ± SD; *N*)
Young	13.3 ± 0.5; 10	13.0 ± 0.0; 10
Old	84.1 ± 8.5; 13	82.1 ± 8.3; 10

Tissue extraction and preparation

Following imaging studies, mice were euthanized via cervical dislocation and brains were flash frozen on dry ice and stored at −80 °C. Tissues were later prepared for steroid analyses and polymerase chain reaction (PCR). All biochemical analyses were conducted in a manner blinded to condition.

Enzyme-linked immunosorbent assay for steroids

Enzyme-linked immunosorbent assay (ELISA) for testosterone and estradiol was carried out as previously described with modifications to accommodate brain tissue [15, 16, 20]. Briefly, MPFC and bilateral striatum (caudate/putamen) were grossly dissected and wet weights were collected. Dissected tissue was homogenized in a 10× volume of PBS using a Pro-200 tissue homogenizer (Pro-Scientific Inc, Oxford, CT). Homogenized tissue was incubated with ice-cold ether in glass borosilicate culture tubes, snap frozen, and evaporated to dryness. Steroid was reconstituted with kit extraction buffer at a 1:20 dilution factor and assayed for testosterone (Neogen Corp., #402510, Lexington, KY) and estradiol (Neogen Corp., #402110) per manufacturer instructions. The testosterone antibody reportedly cross-reacts with testosterone and dihydrotestosterone 100% and nominally with other steroids (< 1%). The estradiol antibody reportedly cross-reacts with 17β-estradiol 100%, testosterone 1%, and nominally with other steroids (< 1%). The intra-assay variance for testosterone was 3.3% and for estradiol was 4.4%.

Expression of *tat* mRNA via polymerase chain reaction

Total RNA of the remaining brain tissue was isolated with TRIzol reagent according to manufacturer's recommendations (Life Technologies, Inc.). RNA was cleaned using the Qiagen RNeasy Mini Prep kit© (Qiagen, Germany; #74104). Synthesis of cDNA was performed with 1 μg of total RNA using a RevertAid First Strand cDNA Synthesis kit (Thermo Fisher Scientific; #K1651). Polymerase Chain Reaction (PCR) was performed with 1 μg of cDNA in a 25 μL final volume containing primers (400 nM) and PowerUp SYBR Green master mix (Thermo Fisher Scientific;

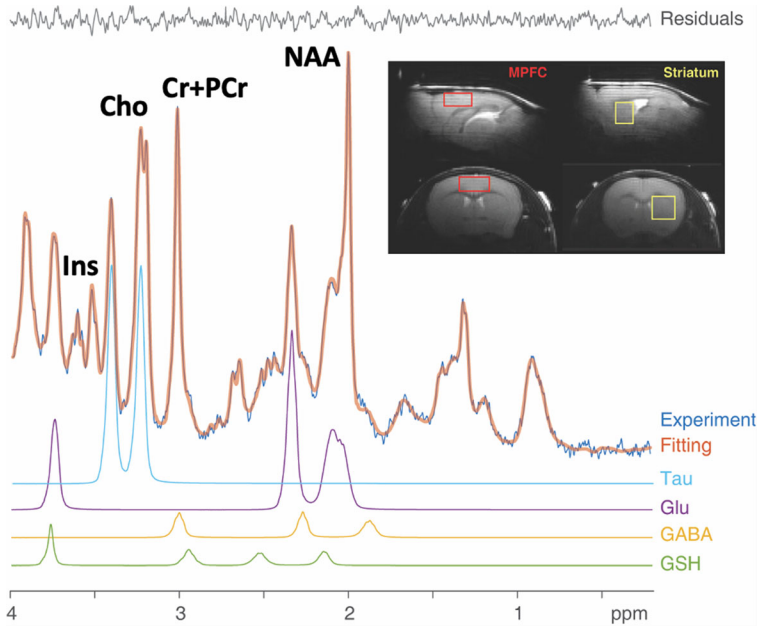

Fig. 1 Sample medial prefrontal cortex (MPFC) MRS scan from an aged Tat-tg mouse. Shown are the original data (light blue trace), spectrum fit (brown trace for the entire spectrum, and fit residuals. To illustrate LC Model peak fitting, taurine (Tau), glutamate (Glu), GABA, and glutathione (GSH) peak fits are shown below the complete spectrum. Additional peaks that were quantifiable with high precision were N-acetylasparate (NAA), creatine + phosphocreatine (Cr+PCr), choline (Cho), and myo-inositol (Ins). Insets: MPFC and striatum MRS Voxels (red and yellow boxes, respectively) overlaid on sagittal (top row) and coronal (bottom row) MRI images (A, anterior; P,posterior; R,right; L, left)

#A25742), using specific primers to *tat* 5′-GGA ATT CAC CAT GGA GCC AGT AGA TCC T-3′ and 5′-CGG GAT CCC TAT TCC TTC GGG CCT GT-3′ and *GAPDH* 5′-GGA AGC TCA CTG GCA TGG C-3′ and 5′-TAG ACG GCA GGT CAG GTC CA-3′. Primers were purchased from IDT (Coralville, IA). Reactions were initiated by denaturation at 94 °C for 2 min, followed by 40 cycles of 94 °C for 30 s, 60 °C for 30 s, 72 °C for 30 s, and one cycle of extension at 72 °C for 7 min. Relative expression of *tat* and *GAPDH* mRNA was quantified via densitometry of 1.8% agarose gels (see Supplementary Information Figure S1) using Fiji [52]. The expression of *tat* was normalized to that of *GAPDH* for quantitative analyses.

Data analyses

MRS data processing was conducted in a blind manner with respect to mouse age and sex. After correcting for frequency and phase shifts, FIDs were summed for metabolite quantification. Spectra were analyzed using the LCModel frequency domain fitting method [53, 54]. The basis set for LCModel was simulated using density matrix simulations [55]. This procedure enabled quantification of metabolites of interest including glutamate (Glu), GABA, glutathione (GSH), N-acetylaspartate (NAA), myo-inositol (mI), and taurine. Metabolites with Cramer Rao Lower Bound (CRLB) fit values exceeding 20% were considered unreliable and excluded from statistical analyses. We used 2-way ANOVAs with Tukey's HSD post hoc tests to determine effects of age and sex (Prism Version 8.4.3, GraphPad Software, LLC). Differences in the brain expression of *tat* and *GAPDH* mRNA and steroid concentrations among aged male and female mice were assessed via Student's two-tailed *t*-tests. Simple linear regressions were utilized to characterize significant associations between all continuous variables. For all analyses, the threshold for statistical significance was set at $p = 0.05$.

Results

Mean ages of mice scanned for this study are shown stratified by sex in Table 1. Within each age group, there were no age differences as a function of sex. With regard to MRS metabolites, we detected effects of age on GABA and taurine levels in MPFC (GABA: $F_{1,39}$=7.7, $p = 0.008$, taurine: $F_{1,39}$=11.7, $p = 0.002$) and striatum (GABA:

$F_{1,39}$=5.3, p = 0.027, taurine: $F_{1,39}$=6.4, p = 0.016), both of which were lower in older Tat-tg mice, as well as in Glu levels in MPFC ($F_{1,39}$=21.6, p < 0.001), which also was lower in older Tat-tg mice (Fig. 2). We quantified the relative magnitudes of age effects on these metabolites by calculating mean Glu and GABA levels in each age group (using weighted-means to account for different numbers of mice in each group), and we found comparable MPFC reductions for Glu (−19.5%) and GABA (−15.5%) in older mice. By contrast, in striatum, we found a small age-related decrease of Glu levels (−4.9%) but an apparently larger effect of age on GABA levels (−27.7%). Because age-matched control mice not expressing Tat were not included in this study, we cannot determine the extent to which these effects are attributable to age or a combination of age plus Tat expression. We also detected an effect of sex on taurine levels in MPFC ($F_{1,39}$=5.6, p = 0.023) and striatum ($F_{1,39}$=4.9, p = 0.033). In MPFC, taurine and GSH were lowest in older female Tat-tg mice (Fig. 2), and we detected an age × sex interaction effect on GSH level ($F_{1,39}$=4.9, p = 0.033). We did not detect effects of age or sex on levels of other MRS metabolites (Supplementary Information Table S1).

PCR confirmed the presence of *tat* mRNA in the brains of aged Tat-tg mice, consistent with transgene leak reported elsewhere [24] (Supplementary Information Figure S1). Expression of *tat* mRNA normalized to *GAPDH* did not significantly differ by sex (Table 2) nor did it correlate with any other continuous variable. However, *GAPDH*-normalized *tat* mRNA expression was higher in our aged Tat-tg mice (~4.75-fold greater) than the low level expression reported in uninduced young adult Tat-tg mice [46].

Testosterone concentrations were greater among aged males compared with aged females in MPFC [t(18) = 3.94, p = 0.001] and striatum [t(18) = 3.54, p = 0.002; Table 3]. In aged males, greater age predicted lower testosterone content in MPFC [β = −0.45, t(18) = −2.15, p < 0.04] and striatum [β = −0.53, t(18) = −2.67, p < 0.02], an effect not observed in females (Fig. 3). No significant differences were observed in brain estradiol or the estradiol/testosterone ratio (an indicator of aromatization). No additional correlations between steroid content and other study measures were observed.

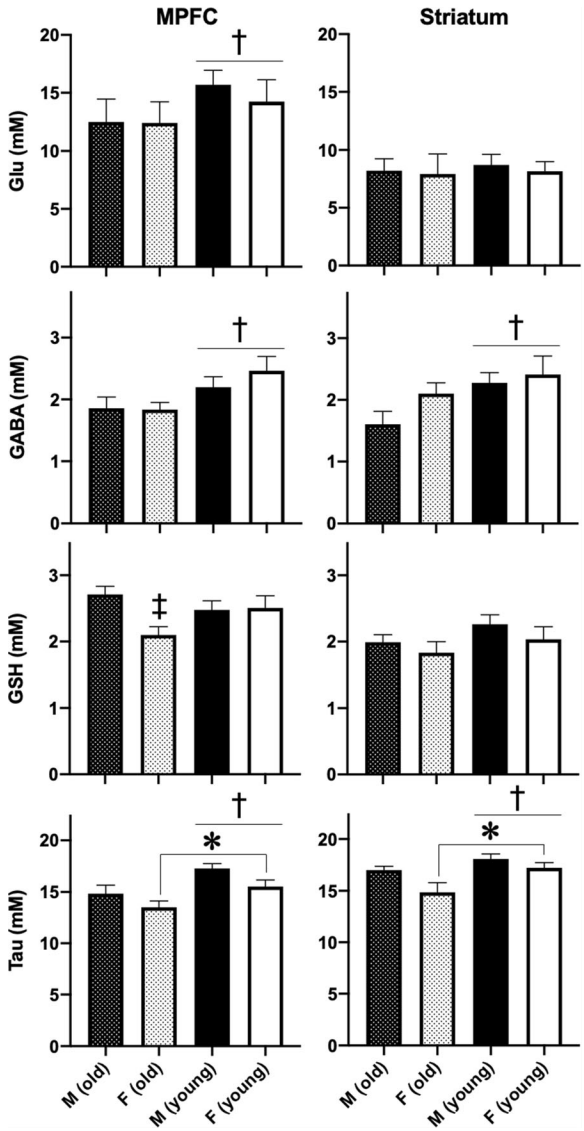

Fig. 2 Effects of age and sex on medial prefrontal cortex (MPFC, left column) and striatal (right column) metabolite levels for glutamate (Glu), GABA, glutathione (GSH), and taurine (Tau). Shown are means + SEMs of 10-13 observations per group. GABA and taurine levels were lower in both brain regions in older mice and MPFC Glu levels were lower in older mice. MPFC GSH levels were lower in older female mice and taurine levels in both brain areas were lower in female mice. Asterisk indicates a main effect of sex. Dagger indicates a main effect of age. Double dagger indicates an interaction wherein older females differ from older males, p < 0.05

Discussion

We found lower MPFC GABA, Glu, and taurine levels and lower striatal GABA and taurine levels in aged uninduced Tat-tg mice. In MPFC, we also detected effects of sex, finding lower GSH and taurine levels in female versus male mice. Similarly, in striatum, taurine levels were lower in females

Table 2 Relative expression of *tat* mRNA in aged male (n = 9) and female (n = 12) Tat-transgenic mice. PCR blots depicted in Supplementary Information Figure S1

mRNA expression	Aged ♀	Aged ♂
tat relative to *GAPDH* (mean, *tat*/*GAPDH* ± SD)	1.67 ± 0.35	1.95 ± 0.55

than males. Several of these effects parallel MRS changes identified in a longitudinal study of genetically similar C57BL/6J mice [38]. In that study, striatal GABA, Glu, GSH, and taurine declined in C57BL/6J mice aged 18 months (within the age range of the older mice we studied) as did cortical Glu [38]. Additionally, female C57BL/6J mice exhibited lower cortical and striatal taurine levels than males [38]. By contrast, we detected age effects in Tat-tg mice not reported in 18-month-old C57BL/6J mice [38] including cortical GABA, GSH, and taurine levels. While these apparent differences could result from a cohort effect in our study or from the fact that we acquired cortical MRS data from an anterior MPFC voxel versus a more posterior cortical voxel scanned in the C57BL/6J mouse study [38], it also is possible that aged Tat-tg mice experience more cortical MRS abnormalities than aged wild-type C57BL/6J mice.

A strain difference is plausible because unlike C57BL/6J mice, Tat-tg mice experience chronic Tat transgene leak and Tat mRNA and protein exposure sufficient by 1 year of age to promote neuroinflammation, brain structural abnormalities, pre- and postsynaptic abnormalities, and axonal damage [24]. Similarly, Dox induction of Tat expression in Tat-tg mice for 1 year causes robust brain and behavioral effects including astrocyte activation in cortex and striatum, impaired long- and short-term memory, and motor dysfunction [17]. Thus, prolonged exposure to Tat protein in Tat-tg mice either by Tat transgene leak or by Dox induction affects brain structure, function, and behavior. That being said, our study lacks controls that would have allowed us to deconvolve the effects of age and Tat expression, a goal for future investigations. Below, we discuss potential implications of each of the MRS metabolite abnormalities we detected.

GABA and glutamate

Glu and GABA are the major excitatory and inhibitory neurotransmitters, respectively. Accordingly, the present findings in striatum that GABA but not Glu levels were lower in aged mice (Fig. 2) could suggest that older Tat-tg mice maintain lower striatal inhibitory tone, a possibility consistent with striatal electrophysiology abnormalities found in Tat-tg mice [41, 43]. By contrast, Glu and GABA levels both were lower in MPFC in older mice by comparable degrees, perhaps reflecting minimal change in excitatory/inhibitory balance with aging. However, Glu and GABA exist in neurotransmission and metabolic pathways [56] that cannot be distinguished with the in vivo proton MRS methods we used. Accordingly, the differential effects of age on MPFC and striatal Glu and GABA we report could reflect neurotransmission and/or metabolic abnormalities that could manifest differently in these brain regions. We suspect that abnormal metabolism may contribute to the present findings because Tat impairs mitochondrial function and metabolism [19, 26–28, 34] and thus could affect Glu and GABA levels in metabolic pools. Future studies employing in vivo proton MRS along with phosphorus (^{31}P) MRS could help to resolve this issue by concurrently characterizing Tat's effects on mitochondria and metabolism [57].

Table 3 Testosterone (ng/g) and estradiol (ng/g) content in the medial prefrontal cortex (MPFC) and striatum of aged male (n = 8) and female (n = 12) Tat-transgenic mice

	MPFC		Striatum	
	Aged ♀	Aged ♂	Aged ♀	Aged ♂
Testosterone (mean, ng/g ± SD)	0.13 ± 0.14	1.04 ± 0.80*	0.08 ± 0.14	1.04 ± 0.94*
Estradiol (mean, ng/g ± SD)	0.019 ± 0.014	0.013 ± 0.024	0.024 ± 0.023	0.019 ± 0.018
Estradiol:testosterone ratio (mean ± SD)	27.0 ± 67.9	1.3 ± 1.7	11.0 ± 15.4	1.9 ± 2.0

*Greater than females, Student's t-tests p = 0.001–0.002

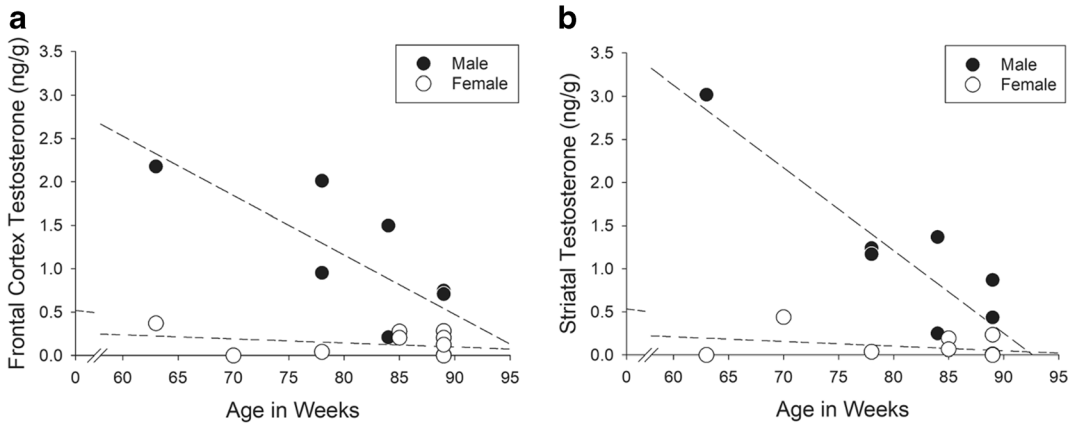

Fig. 3 Age was inversely associated with testosterone concentration in **a** frontal cortex ($r_{male} = -0.76$, $R^2 = 0.58$, $p = 0.03$; $r_{female} = -0.30$, $R^2 = 0.09$, *not significant*) and **b** striatum ($r_{male} = -0.90$, $R^2 = 0.82$, $p = 0.002$; $r_{female} = -0.34$, $R^2 = 0.12$, *not significant*) of males (closed circles) but not females (open circles). Dotted lines depict simple linear regressions

Glutathione

GSH is the most abundant endogenous small molecule antioxidant and it plays a key role in buffering excess oxidative stress [58]. GSH levels also are depleted by inflammatory mediators including lipopolysaccharide, IL-1β, and TNFα [59–61], which increase production of reactive oxygen species normally neutralized by GSH [62]. Accordingly, the lower MPFC GSH levels we found in females, driven mainly by lower levels in older females (Fig. 2), could reflect higher levels of oxidative stress and/or inflammation in these mice. Estrogen exerts antioxidant [63–66] and anti-inflammatory [66, 67] effects, and thus estrogen declines could result in GSH depletion, an effect reported in rats after abrupt estrogen depletion via ovariectomy [64–66, 68]. It also is plausible that chronic Tat transgene leak in older Tat-tg mice may contribute to lower GSH levels because Tat accelerates decline of circulating estrogen levels [15] and it induces inflammation, oxidative stress, and abnormal GSH levels [6, 8, 14, 21–25, 44, 69–71]. Accordingly, the combined effects of aging and Tat transgene leak in older female Tat-tg mice could more substantially deplete GSH, resulting in the age × sex interaction effect we detected for MPFC GSH levels. An MPFC GSH abnormality could have functional implications as the frontal cortex is seminally involved in cognition and behavioral inhibition, both of which are impaired by short- and long-term Tat expression [9, 15, 17, 23].

Taurine

Taurine regulates osmotic balance and cellular volume, it inhibits excitatory glutamatergic N-methyl-D-aspartate receptor signaling, it facilitates mitochondrial protein syntheses, and like GSH, taurine exerts antioxidant and anti-inflammatory effects [72–74]. In MPFC, taurine declines with aging were greater in females than in males, which could amplify inflammation and oxidative stress and contribute to the relatively large MPFC GSH decrements we found in older female Tat-tg mice. The cortical and striatal taurine declines detected presently in older mice parallel postmortem findings in rat cortex and striatum [75]. By contrast, cortical taurine levels quantified by MRS remained stable in C57BL/6J mice up to 24 months of age [38]. This apparent study difference, like that noted above for cortical GSH, may be attributable to a cohort effect, to the fact that our MRS data are from a frontal cortex voxel versus a more posterior cortex voxel scanned in the C57BL/6J mouse study [38], and/or to the combined effects of aging plus prolonged Tat transgene leak in Tat-tg mice, which could exacerbate taurine depletion. An effect of Tat transgene leak also is plausible given that induction of Tat expression induces cortical edema in young Tat-tg mice [46], that Tat likely causes edema in older female Tat-tg mice [15], and that edema triggers cellular taurine loss to counter extracellular hypoosmolality [73]. Because estrogen upregulates taurine transporter activity [76] and reduces taurine efflux in an estrogen receptor-dependent manner consequent to glutamate excitotoxicity [77], estradiol declines in older female

mice also may contribute to the especially low MPFC and striatal taurine levels we found in older female Tat-tg mice (Fig. 2).

Striatal taurine declines in aged rats have been linked to abnormal striatal dopamine levels [45, 78] and to impaired spatial learning [78], the latter of which was found after Dox induction of Tat expression in young and old Tat-tg mice [9, 10, 13, 15, 17]. Given that Tat impairs mitochondrial function and induces excess oxidative stress and inflammation [6, 8, 14, 20–25, 44, 69–71] and that taurine preserves mitochondrial health [72–74], the cortical and striatal taurine declines we found in aged Tat-tg mice could exacerbate mitochondrial dysfunction, oxidative stress, and inflammation in these brain areas. Because taurine supplementation in aged mice improved spatial memory as well as Glu and GABA levels [79], age-related taurine depletion could contribute to Glu, GABA, and spatial memory impairments detected in aged Tat-tg mice as observed presently and elsewhere [15, 17].

The present findings have potential translational value based on recent demonstrations in elderly women that short-term dietary taurine supplementation reduced systemic inflammation while dietary taurine in combination with exercise improved cognition [80]. Both effects may have resulted in part from correcting low systemic taurine levels in older individuals [81]. By opposing several deleterious effects induced by Tat protein and by HIV, dietary taurine supplementation could have promise as a novel adjunct treatment for older individuals living with HIV. To our knowledge, taurine supplementation has not been tested either in Tat-tg mouse models or in individuals with HIV. Similarly, sex steroid hormones and their analogs exert pleiotropic beneficial effects and attenuate Tat-mediated neurological impairments ([20, 27, 82], possibly by inhibiting inflammation and oxidative stress and increasing cellular GSH and taurine levels [63–67, 76, 77]. The low brain testosterone levels we found in male Tat-tg mice as a function of age are consistent with reports of lower circulating sex steroid levels among HIV^+ individuals [31, 32, 83, 84]; albeit, levels within the central nervous system cannot typically be assessed clinically. Accordingly, in future studies, we plan to test whether supplementation with taurine, sex steroid hormones, or sex steroid hormone analogs normalizes MRS metabolites in aged murine models of HAND. Such studies are needed given that more than half of the US population living with HIV is middle-aged or older [1] and that Tat protein expression endures and may even increase during cART [5–7]. These findings help position Tat as a potentially important therapeutic target in older individuals living with HIV.

Limitations

A major limitation to this study is that we did not scan age-matched wild-type (C57BL/6J) mice to determine whether the MRS effects we detected as a function of age and sex in Tat-tg mice differ from effects of age and sex in wild-type mice or in other controls (e.g., Tat(-)-tg mice). Also, we did not directly measure oxidative stress or inflammation. Thus, we cannot deconvolve individual effects of age or *tat* mRNA expression on outcomes and we cannot definitively conclude that MRS metabolite abnormalities reflect oxidative stress or inflammation. Further, we did not quantify sex steroid hormone levels or *tat* mRNA levels in young Tat-tg mice in this this study so we cannot determine whether the MRS differences we detected as a function of age could be driven by age-related sex steroid abnormalities, Tat expression increases, or by a combination of these effects. Yet, prior studies in older Tat-tg mice indicate that nearly two-thirds of 18-month-old female mice are peri- or post-estropausal and exhibit sex steroid hormone imbalances [15] and that Tat transgene leak is considerable by 1 year of age [24]. Together, these findings support the possibility that sex steroid abnormalities and Tat transgene leak contribute to the present MRS findings. These issues will be addressed directly in our future studies. Notwithstanding these limitations, the present data indicate that age and sex differences in several MRS metabolites are apparent in uninduced Tat-tg mice including for metabolites that reflect oxidative stress and inflammation (GSH and taurine), neural excitation, inhibition, and metabolism (Glu and GABA), and osmolality (taurine). These MRS metabolites may serve as useful noninvasive biomarkers in future prospective studies of aged Tat-tg mice including those evaluating novel treatments for HIV.

Supplementary Information The online version contains supplementary material available at https://doi.org/10.1007/s11357-021-00354-w.

Availability of data and material Data will be made available to qualified researchers upon request.

Author contributions Drs. Paris and Kaufman conceptualized the study.

Drs. Chen, Du, and Ms. Qrareya, Mahdi, and Mr. Anderson acquired the data.

Drs. Paris, Chen, Du, and Kaufman, and Ms. Qrareya, Mahdi, and Mr. Anderson analyzed the data.

Drs. Paris, Du, and Kaufman interpreted the data.

Drs. Paris and Kaufman drafted the manuscript.

All authors reviewed, provided edits, and approved of the manuscript final version.

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Declarations

Conflict of interest The authors declare no competing interests.

Ethics approval The animal research included in this study was conducted after review and approval by the McLean Hospital Institutional and Animal Care Use Committee (IACUC).

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