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Conditional Human Immunodeficiency Virus Transactivator of Transcription Protein Expression Induces Depression-like Effects and Oxidative Stress

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Abstract

Background—The prevalence of major depression in those with HIV/AIDS is substantially higher than in the general population. Mechanisms underlying this comorbidity are poorly understood. HIV-transactivator of transcription (Tat) protein, produced and excreted by HIV, could be involved. We determined whether conditional Tat protein expression in mice is sufficient to induce depression-like behaviors and oxidative stress. Further, as oxidative stress is associated with depression, we determined whether decreasing or increasing oxidative stress by administering methylsulfonylmethane (MSM) or diethylmaleate (DEM), respectively, altered depression-like behavior.

Methods—GT-tg bigenic mice received intraperitoneal saline or doxycycline (Dox, 25–100 mg/kg/day) to induce Tat expression. G-tg mice, which do not express Tat protein, also received Dox. Depression-like behavior was assessed with the tail suspension test (TST) and the two-bottle saccharin/water consumption task. Reactive oxygen/nitrogen species (ROS/RNS) were assessed ex

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Disclosures

GDV has an ownership interest in EXQOR Technologies Inc. that owns patent rights to clathrin nanotechnologies. MJK receives funding from the National Institute on Drug Abuse and National Institute of Mental Health. He is an inventor of a technology using xenon gas to treat aversive memory disorders including Post-Traumatic Stress Disorder, which has been licensed by Nobilis Therapeutics, Inc. MJK also currently holds leadership roles in the College on Problems of Drug Dependence. All other authors report no biomedical financial interests or potential conflicts of interest.

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vivo. Medial frontal cortex (MFC) oxidative stress and temperature were measured in vivo with 9.4-Tesla proton magnetic resonance spectroscopy (MRS).

Results—Tat expression increased TST immobility time in an exposure-dependent manner and reduced saccharin consumption. MSM decreased immobility time while DEM increased it in saline-treated GT-tg mice. Tat and MSM behavioral effects persisted for 28 days. Tat and DEM increased while MSM decreased ROS/RNS levels. Tat expression increased MFC glutathione levels and temperature.

Conclusions—Tat expression induced rapid and enduring depression-like behaviors and oxidative stress. Increasing/decreasing oxidative stress increased/decreased, respectively, depression-like behavior. Thus, Tat produced by HIV may contribute to the high depression prevalence among those with HIV. Further, mitigation of oxidative stress could reduce depression severity.

Keywords

HIV; glutathione; magnetic resonance spectroscopy; major depression; oxidative stress; Tat; transgenic mice

INTRODUCTION

Major depression is among the most common psychiatric comorbidities in those with HIV-1/AIDS, occurring in 30–50% of this population (1,2). This rate substantially exceeds the 11% depression prevalence in the general population (3). Major depression in HIV+ individuals is associated with poor antiretroviral therapy compliance (4–7), increased morbidity (8,9), and accelerated mortality (8–10). Accordingly, advancing our understanding of factors driving the high depression comorbidity among people with HIV may lead to treatments that reduce depression, improve treatment compliance, and reduce morbidity and mortality.

Mechanisms underlying the HIV-1/depression comorbidity have yet to be elucidated. However, virally-produced proteins, including HIV-transactivator of transcription (Tat) protein, may be involved. Tat protein is produced and excreted by HIV-1 virus, including that persisting in reservoirs during antiretroviral therapy. Tat protein or antibodies to newly-excreted Tat protein are detectable in cerebrospinal fluid or blood in 40–50% of patients (11–12), and, over the course of a year, in nearly 90% of antiretroviral therapy-treated patients (11). As infusion of Tat protein acutely increased depression-like behaviors in mice (13) and conditional Tat protein expression induced reward deficits in mice, possibly reflecting anhedonia, a core feature of depression (14), Tat protein could increase vulnerability for depression.

Tat triggers a number of biological effects but its capacity to increase oxidative stress (15–30) could catalyze symptoms of depression. Increased oxidative stress has been associated with behavioral depression in humans (31–37) and animals (38–42). Further, oxidative stress mitigation reduces depression severity in humans (33,43–45) and in animal models (38–42,46,47).

To further investigate whether Tat increases depression-like behavior and whether oxidative stress is involved, we used GT-tg bigenic mice, which contain a doxycycline (Dox)-inducible astrocyte-specific promoter enabling conditional Tat protein expression (48). These mice initially were constructed by crossing Teton-GFAP with TRE-Tat86 mice, to obtain GT-tg bigenic mice (48). Dox treatment induces Tat protein expression in these mice at concentrations of 0.1–0.9 ng/ml in vitro (48), comparable to cerebrospinal fluid Tat concentrations detected in antiretroviral therapy-treated HIV patients (12). Prior studies of Dox-induced Tat-expressing mice have reported cellular (48–55), brain structural (56), and behavioral or cognitive (14,49,50,52–55,57–60) abnormalities. However, to date, no studies have reported on whether controlled Tat protein expression induces depression-like behavior on tasks commonly used to assess such behavior in mice.

Accordingly, we used the tail suspension test (TST) and the two-bottle (saccharin/water) consumption test to determine whether Tat expression induces depression-like behavior. Both tests have been used extensively in mice to characterize depression-like behavior and antidepressant effects (13,38,39,42,46,47,61–64). We also tested whether pretreatment with the antioxidant methylsulfonylmethane (MSM), which mitigates Tat-induced oxidative stress in neuronal culture (30), or pretreatment with the pro-oxidant diethyl-maleate (DEM), which conjugates with and rapidly depletes brain and peripheral glutathione levels (65,66), altered TST behavior. We hypothesized that Tat expression would promote depression-like behaviors and that MSM and DEM would reduce and enhance, respectively, depression-like behavior.

To confirm that Tat protein expression, MSM, and DEM altered cerebral oxidative stress, we measured postmortem levels of whole brain reactive oxygen species/reactive nitrogen species (ROS/RNS). We hypothesized that Tat and DEM would increase ROS/RNS levels and that MSM would reduce ROS/RNS levels.

Lastly, we conducted 9.4 Tesla proton magnetic resonance spectroscopy (MRS) in separate groups of GT-tg bigenic mice to test whether Tat protein altered medial frontal cortex (MFC) glutathione (GSH) levels. MFC was selected for study because it is involved in major depression (67–69). GSH is the principal endogenous antioxidant molecule (70). GSH levels rise slowly with aging, possibly to counteract age-associated increases in cortical oxidative stress (71). However, MRS studies in people with major depression reported higher anterior cingulate cortex GSH levels and that GSH level was associated with depression symptom severity (32). Similarly, in people at risk for major depression, stabilization of thalamic GSH levels with fatty acid supplements was associated with lower depression severity than found in placebo-treated subjects, in whom GSH levels increased (33). While we are unaware of any MRS studies in HIV+ subjects or in animal models reporting on brain GSH levels, postmortem thalamic GSH levels were elevated in older HIV-1 transgenic rats (72), which exhibit depression-like behavior (64). Accordingly, we hypothesized that Tat protein expression would increase MFC GSH levels. We also characterized Tat's effects on MFC temperature, which can be determined in proton spectra (73). Because Tat increases Tumor Necrosis Factor alpha levels (13,74–76), which increase expression of mitochondrial uncoupling proteins (77–81) that dissipate oxidative stress in the form of heat (82), we hypothesized that Tat protein expression in GT-tg mice would increase MFC temperature.

METHODS AND MATERIALS

Subjects

GT-tg bigenic mice (47) and G-tg mice, in which the Tat transgene was outbred from GT-tg mice, were produced at the University of Florida (UF). C57BL/6J mice (Jackson Laboratories), a parent strain of GT-tg mice, also were used in ROS/RNS studies. Male mice, 8–13 weeks of age, were studied. Subjects were maintained on a 12:12 light:dark cycle and had *ad libitum* food and water access. For imaging studies, GT-tg mice were transported from UF to McLean Hospital via commercial courier and acclimated to the McLean Hospital vivarium for at least one week prior to initiating study procedures. Animal procedures were approved by Institutional Animal Care and Use Committees at both institutions and conformed to National Research Council guidelines (83). Supplemental Information Table S1 summarizes studies that were conducted.

Drug Treatments

Tat protein expression was induced in GT-tg bigenic mice by intraperitoneal administration of Doxycycline hyclate (Dox, Sigma-Aldrich, St. Louis, MO). Dox was prepared fresh daily by dissolving in vehicle (sterile 0.9% saline) to the concentration enabling administration of 0.1 ml per 10 g body weight, and protected from light. Methylsulfonylmethane (MSM, Sigma-Aldrich), which has a half-life in rodents of about 12 hours (84), was administered intraperitoneally at 100 mg/kg/day, 20 minutes before GT-tg mice were administered saline/Dox, adapted from (85). Diethyl maleate (DEM, Sigma-Aldrich), which rapidly depletes GSH with a half-life of about 15 hours (64), was given intraperitoneally for one or two days. DEM was administered at a dose of 34.8 μ M in 0.25 ml saline, and in pretreatment studies was given 30 minutes before saline or Dox based on prior studies in mice (65,86).

Tail Suspension Test (TST)

A TST protocol modified from (61) was conducted. Mice were supported by a cage lid and their tails were secured with laboratory tape to a horizontal surface 18 inches above the floor. Prior to taping, a small, clean plastic disc was placed over tails to prevent tail climbing, which confounds the assay. Then, cage tops were slowly removed to suspend mice for 5 minutes. Time spent immobile (the adoption of a stationary posture) was measured by treatment-blinded observers. TST tests were conducted in mice after 1 or 7 days of treatments, and again 7, 14, and 28 days after terminating drug treatments to assess for enduring treatment effects.

Saccharin Consumption Task

The saccharin consumption protocol was modified from (62). Individually-housed mice were habituated for 4 days to drink from two 100-mL bottles containing water. On day 5, the second bottle was filled with saccharin (0.2% w/v). To control for bottle position preference, bottle position was changed every 24 h at 1200. Body weight (g) and fluid intake (g and mL) were measured daily.

Magnetic Resonance Imaging (MRI) and Spectroscopy (MRS)

Imaging was performed at McLean Hospital in isoflurane-anesthetized (1.5–2%) GT-tg mice after 7 days of intraperitoneal saline or 100 mg/kg/day Dox injections. We used a 9.4 Tesla Direct Drive Scanner (Agilent, Inc.), a horizontal 60 mm inner diameter gradient insert with a strength of 100G/cm, and a custom-made volume coil for MRI and MRS. Vital signs were monitored (Small Animal Instruments, Inc.) and maintained by adjusting isoflurane concentration and warm air flow.

Fast-spin-echo scans were acquired coronally (TR (repetition time)=2.1 seconds, TE (echo time)=60 milliseconds, acquisition matrix=128×128) to enable MRS voxel (2.0×3.0×2.5 mm=15 µl) positioning over MFC (Figure 1). We used an ultrashort TE stimulated echo acquisition mode (STEAM) sequence (87) with TR/TE=4 seconds/3 milliseconds, 4096 complex points, 5000 Hz acquisition bandwidth, and 1 ms 90° excitation pulse. FASTMAP automated shimming (88) produced 10–13 Hz water linewidths. VAPOR water suppression (89) was used to acquire water-suppressed partial spectra as 5 blocks of 128 shots, taking 8.5 minutes each, to minimize effects of frequency drift. Traces from each block were added using the 2.0 ppm N-acetylaspartate (NAA) peak to synchronize blocks. Each complete MRS spectrum contained 640 shots (42.5 minutes). Water-unsuppressed spectra (4 shots) were acquired in tandem with each water-suppressed spectrum block using identical parameters, except for water suppression. Water-unsuppressed spectra were used to correct for phase and eddy current distortions, to derive tissue water concentration used to normalize metabolite levels, and to calculate water chemical shifts for MFC temperature measurement (see below).

MRS Data analyses

Water-suppressed spectra were pre-processed using custom-written Matlab code (Mathworks, Inc., vR2012a). Spectra were visually inspected and apodized using a 4 Hz exponential filter, to remove high frequency noise, which improves spectrum quality without affecting metabolite ratios (90). Water-suppressed spectra were corrected for the phase and residual eddy currents (91). Pre-processed spectra were fitted with LC Model to determine metabolite levels and water peak frequencies (92) using a GAVA-simulated basis set (93). A sample fitted water-suppressed spectrum is shown (Figure 1).

Temperature Estimation

MFC temperature was estimated by calculating the chemical shift difference between each partial spectrum N-acetylaspartate (NAA) peak and its paired unsuppressed-water water peak (73). Spectra were processed with 2Hz exponential apodization and the auto peak picker function in MestRenova (v. 9.0) was used to identify water and NAA resonance frequencies. Temperature was estimated by comparing water (H₂O) and NAA chemical shifts (δ) (73):

$$T_{\text{brain}} (^{\circ}\text{C}) = 36.0 - 103.83 \times (\delta\text{H}_2\text{O} (\text{ppm}) - \delta\text{NAA} (\text{ppm}) - 2.6759)$$

ROS/RNS Quantification

Whole-brain ROS/RNS were quantified *ex vivo* per manufacturer's guidelines (OxiSelect fluorescence assay, STA-347, Cell Biolabs, Inc.) in brains from GT-tg mice administered saline/Dox for 1, 7 or 14 days, in mice pretreated with MSM (100 mg/kg i.p.), 10 minutes before saline/Dox for 7 or 14 days, and in C57BL/6J mice administered DEM (34.8 μ M, 250 μ L per 25g, 30% Solutol/Saline) for 1 or 2 days or vehicle (30% Solutol/Saline) for 1 or 5 days. After drug treatments, mice were anesthetized with isoflurane, euthanized by cervical dislocation, brains were extracted and flash frozen in liquid nitrogen, and stored at -80°C . Tissue was homogenized to 25 mg/mL concentration in phosphate buffered saline (pH 7.2) with a QSonica sonicator. Homogenate aliquots (1 mL) were centrifuged (5 min x 6000 rpm, Corning® LSE™ Mini-Microcentrifuge) and supernatant was immediately assayed with a Synergy H1 Multi-mode reader (BioTek Instruments, Inc.).

Statistical analyses

Behavioral and ROS/RNS statistical analyses—Data are presented as mean $\pm$ SEM. Behavioral data were analyzed via univariate ANOVA (Prism 7 GraphPad Software). A one-way ANOVA was used with the between-subject factor of Dox dose (0–100 mg/kg/d, 7 days) on TST immobility time in GT-tg mice. Additional group differences were examined by two-way ANOVAs with factors of duration (1 and 7 days) x treatment (saline or Dox, 100 mg/kg/d) and their interaction, or with factors of treatment (saline or Dox) x mouse strain and their interaction. Post hoc testing with Tukey's HSD was used to compare all means. Saccharin consumption was analyzed by two-way ANOVA with factors time x treatment). Sidak's Multiple Comparison *post hoc* tests were used to control family-wise error rates and assess group differences following main effects. A three-way ANOVA (SPSS software, IBM) was used to analyze effects of Tat (Dox or saline treatment), antioxidant treatment with each condition and post-treatment testing day as factors. Additional two-way ANOVAs were run to compare simple main effects of all interactions between factors, with Tukey's HSD *post hoc* testing as appropriate. A one-way ANOVA was used to analyze effects of DEM exposure. One-way ANOVAs also were used to analyze ROS/RNS data with factors of time or mouse strain, and Tukey's HSD *post hoc* test. Cohen's *d* values were used to assess effect size magnitudes, where appropriate. Student-Neuman Keuls *post hoc* test was used in one case of ROS/RNS analysis (with C57BL/6J mice) to compensate for an uneven sample size. Analyses were considered significant when $p < 0.05$.

MRS statistical analyses—MRS metabolite differences were assessed using two-sample, two-tailed t-tests (Stata v12, StataCorp.). Temperature effects were assessed using repeated measures two-way ANOVA with treatment and partial acquisition as the between- and within-subjects factors, respectively, and with Bonferroni correction. GSH levels were compared to brain temperature via a Pearson correlation analysis, as detailed below. Brain temperature analyses were conducted with Prism 7 (GraphPad Software).

Data values two standard deviations from group means were excluded: 2 measurements were excluded from MRS, 1 from ROS/RNS measurements, 5 from brain temperature and 18 from TST measurements. Two of 16 Dox-treated mice died between days 13–14, and 3 died

between days 15–16 of the saccharin consumption task, resulting in 14 missing data points from these later testing days.

RESULTS

Tat expression in GT-tg bigenic mice for 7 days increased day 8 TST immobility time in a dose-related manner ($F_{(3,66)}=3.20$, $p=0.03$; 1-way ANOVA with factor: dose Dox, Tukey's HSD, Figure 2). Tat expression for only one day increased TST immobility time versus saline-treated GT-tg mice ($F_{(1,80)}=15.43$, $p=0.0002$; 2-way ANOVA with factor: Dox treatment, with Tukey's HSD; Figure 2), by a magnitude equivalent to that after 7 days of Dox administration ($F_{(1,80)}=0.005$, $p=0.95$; 2-way ANOVA with factor: treatment days; $p=0.73$; Figure 2). By contrast, 7-day Dox treatment of G-tg mice did not increase day 8 immobility time over saline-treated G-tg mice ($p=0.79$, Student's t-test; Figure 2). Effects of strain on day 8 immobility were significant ($F_{(1,77)}=4.76$, $p=0.03$; 2-way ANOVA with factors: treatment x strain), indicating that Tat expression is required to observe immobility increases. Accordingly, subsequent behavioral experiments were conducted in GT-tg mice administered saline (controls) or induced with Dox (100 mg/kg/day, i.p.). Additional groups of GT-tg mice were administered Dox or saline for 7 days and tested for saccharin-flavored water consumption. Tat-expressing mice consumed less saccharin-flavored water (Figure 3). Group differences in saccharin preference became apparent after 1 day and significant after 3 days of Dox treatment ($F_{(1,496)}=4.31$, $p<0.0001$; 2-way ANOVA, Sidak's Test Figure 3). By contrast, Tat expression did not alter water consumption ($F_{(1,496)}=1.13$, $p=0.288$; 2-way ANOVA, Figure 3).

Next, we determined whether MSM pretreatment altered TST immobility time. There was an overall effect ($F_{(15,360)}=12.8$, $p<0.0001$; 3-way ANOVA with factors of Tat exposure x drug treatment x testing time after treatment; Figure 4). Replicating and extending initial results, 7-day Dox treatment increased day 8 immobility time, an effect that persisted for up to 28 days after Tat induction was terminated, versus saline-treated controls ($F_{(1,360)}=28.5$, $p<0.001$; 2-way ANOVA, factor: Tat exposure; Figure 4). MSM pretreatment had a globally significant effect on immobility time ($F_{(1,360)}=36.1$, $p<0.001$; 2-way ANOVA, factor: drug treatment), reducing day 8 immobility time in Tat-expressing mice ($F_{(1,360)}=13.2$, $p<0.001$; 2-way ANOVA, factors Tat exposure x drug treatment; Figure 4) and for up to 28 days later ($p<0.01$ each *post hoc* test; Figure 4). MSM treatment did not significantly alter day 8 immobility time in saline-treated controls ($p=0.30$; Figure 4a), but it reduced it in saline-treated mice tested 28 days after the last drug treatments ($p<0.05$, Tukey's HSD *post hoc* test; Figure 4). By contrast, a single pretreatment with DEM before saline administration substantially increased day 2 TST immobility time ($F_{(3,91)}=3.21$, $p=0.03$; 1-way ANOVA, Tukey's HSD; Figure 4) by the same magnitude as in Dox-only treated mice (87.9 ± 6.6 vs 89.4 ± 5.4 s, respectively). However, DEM pretreatment before Dox administration did not further increase immobility time in Tat-expressing mice ($p=0.99$; Figure 4).

Saline treatment for 7 days resulted in statistically equivalent ROS/RNS levels in GT-tg and G-tg mice (N 's=12/group) and in C57BL/6J mice administered vehicle (N =8) for 5d (3940 ± 402 vs. 3860 ± 282 , 3850 ± 384 , respectively; $F_{(2,29)}=0.02$, $p=0.98$; 1-way ANOVA, Tukey's HSD). Dox administration for 1, 7, or 14 days (N 's=10/group) increased ROS/RNS

levels in GT-tg mice (4990 ± 379 , 4340 ± 200 and 5010 ± 292 , respectively), an effect attaining significance at 14 days ($p=0.04$, Effect size (Cohen's d) = 1.16). By contrast, G-tg mice administered Dox for these durations (N 's=10/group) exhibited no significant ROS/RNS increase (3980 ± 460 , 3980 ± 318 and 4220 ± 273 , respectively). Separate groups of GT-tg mice were pretreated for 7 or 14 days with MSM 10 min before saline ($N=10$ for 7d and $N=12$ for 14d treatment groups) or Dox ($N=12$ for both 7 and 14d treatment groups). MSM decreased ROS/RNS in saline-treated animals at both time points (2900 ± 199 and 2760 ± 104 , respectively; $F_{(3,40)}=6.57$ $p=0.001$; 1-way ANOVA w/Student-Newman-Keuls *post hoc* Method) and abolished Tat-induced ROS/RNS increases at both time points (2680 ± 101 and 2440 ± 116 , respectively; $F_{(3,39)}=50.1$, $p<0.001$; 1-way ANOVA, Tukey's HSD). In C57BL/6J mice, DEM treatment for 1 ($N=7$) or 2 ($N=13$) days increased ROS/RNS levels (4680 ± 631 and 5520 ± 376 , respectively; $F_{(3,30)}=5.16$, $p=0.005$; 1-way ANOVA, Tukey's HSD) to a degree equivalent to those detected in GT-tg mice after prolonged Tat exposure.

Dox administration to GT-tg mice for 7 days increased MFC GSH/water ratios, which averaged 130% of saline controls ($t=2.98$, $p=0.006$; Table 1). Voxel water content did not differ by treatment (Table 1). Thus, the GSH/water ratio difference is attributable to a group difference in GSH level. Dox-treated mice also exhibited higher MFC temperature than saline controls ($F_{(1, 22)}=5.42$; $p=0.030$; Figure 5) and there was an effect of time, e.g., partial acquisition order ($F_{(4, 88)}=2.65$; $p=0.038$). Post-hoc testing revealed that temperature was elevated only during the first MRS partial acquisition block ($p=0.036$). Accordingly, we tested for an association between first partial acquisition temperatures and GSH/H₂O ratios and found a positive correlation between these measures ($r=0.47$, $p=0.024$; Figure S1).

DISCUSSION

Conditional Tat protein expression rapidly increased depression-like behaviors with near-maximal effects achieved after one day of Tat expression. Dox treatment had no effect in G-tg mice, further implicating Tat protein in depression-like behaviors. Tat's rapid effects in GT-tg mice are consistent with reports that intracerebroventricular Tat infusion induces depression-like behaviors within 24 hours (13) and that conditional Tat expression rapidly elevates intracranial self-stimulation reward thresholds (14). Tat's depression-like effects in the TST persisted for up to 28 days after terminating Dox treatments. As Tat levels return to baseline within 14 days of terminating Dox exposure in GT-tg mice (57), Tat's depression-like effects appear to outlive its detectable expression by at least 2 weeks. If Tat protein exerts similar effects in HIV-infected individuals including in those taking antiretroviral therapy, in whom new Tat protein is sporadically produced and excreted by HIV reservoirs (11), this could contribute to the high prevalence of major depression in those with HIV.

Tat's depression-like effects were blunted by MSM. In neuronal culture, MSM substantially neutralized Tat-induced oxidative stress and mitigated GSH depletion (30). Consistent with these effects, MSM pretreatment prevented Tat-induced ROS/RNS accumulation in Dox-induced GT-tg mouse brain. This suggests that Tat-induced oxidative stress promotes depression-like behavior. Twenty-eight days after terminating treatments, TST immobility time was lower in MSM/saline-treated mice, suggesting an antidepressant-like effect of MSM. This is consistent with reports indicating that antioxidants can reduce depression-like

behaviors in animals (38–42,46,47,63) and can improve symptoms of major depression in humans (33,43–45).

A single pretreatment with DEM, which rapidly depletes brain and peripheral GSH levels (65), increased immobility time in saline-treated GT-tg mice, indicating that GSH depletion by itself has rapid depression-like effects. By contrast, a single DEM pretreatment in Dox-treated GT-tg mice did not further increase TST immobility time. In vitro studies of ROS/RNS levels confirmed that Tat induction and DEM increased and MSM decreased oxidative stress, respectively. Collectively, these data support the hypothesis that both Tat and DEM induce depression-like behavior by increasing, and that MSM reduces depression-like behavior by decreasing, oxidative stress. A limitation of our MSM and DEM studies is that we only tested a single dose of these agents at single pretreatment time points, and therefore it is possible that different pretreatment conditions (doses/timings/durations) might yield different effects. While outside the scope of the present study, future testing will examine these relationships.

In MRS studies, conditional Tat protein expression for 7 days increased MFC GSH levels. No other MRS metabolite changes were apparent, suggesting that the GSH increase is a selective effect of Tat expression. Since GSH is the principal endogenous antioxidant molecule (70), it might be expected that the higher GSH levels we detected reflect lower rather than higher oxidative stress levels. However, postmortem and in vivo imaging studies in healthy humans and animals indicate that brain GSH levels are relatively stable in mature mice until late adulthood/senescence, at which point GSH may increase or decrease in different brain regions (71,94–98). Thus, our observation of increased GSH levels in young adult (8–13 weeks old) Tat-expressing mice over the relatively short interval studied (8 days in MRS studies) likely indicates a pathophysiological state induced by Tat protein. As noted above, Tat protein induces oxidative stress and pro-oxidants can increase brain GSH levels (99). Elevated GSH levels also have been reported in animal models of other disorders involving oxidative stress such as Huntington's Disease, and were interpreted to reflect an endogenous compensatory response to oxidative stress (100,101). Accordingly, we hypothesize that the Tat-induced GSH increase we detected with MRS represents a compensatory response to Tat-induced oxidative stress. Apparently, this response is insufficient to overcome oxidative stress or depression-like behavior over the time frame we studied, the latter of which persisted long after Tat protein induction was terminated and after elevated Tat protein levels normalize (57,60).

Among its signaling properties, Tat protein increases Tumor necrosis factor alpha expression (13,49,74,76) which in the short-term can increase expression of the antioxidant response element transcription factor Nuclear factor-erythroid 2-related factor 2 (Nrf2), resulting in increased GSH production (102). Tat protein also increases Nrf2 expression and activity through the activation of glutamatergic N-methyl-D-aspartate (NMDA) receptors and the enzyme spermine oxidase (103). Thus, the GSH increase we detected could be mediated by multiple mechanisms. Future MRS studies in GT-tg bigenic mice administered Dox for longer periods and DEM or MSM at different doses and durations are needed to determine which mechanisms drive Tat-induced GSH changes and over what time courses GSH increases occur.

Conditional Tat protein expression also increased MFC temperature. Post-hoc analysis revealed that temperature was higher only transiently, during the first partial MRS spectrum. This may be a consequence of isoflurane anesthesia, which substantially increases cerebral perfusion (104–106), a primary mechanism for cooling the brain (107). Thus, sustained cerebral cooling by isoflurane-enhanced perfusion could normalize MFC temperature in Tat-expressing mice in later partial spectra. The temperature increase we detected could result from neuronal hyperactivity (107–109), inflammation (110,111), and/or oxidative stress, the latter of which increases expression of heat-generating mitochondrial uncoupling proteins (77–82). Tat induces neuronal hyperexcitability (112,113), inflammation (13,49,114–120), and as noted above, oxidative stress. Our finding of a strong correlation between initial brain temperature and GSH level (Figure S1) suggests that oxidative stress in part mediated the temperature effect.

Oxidative stress mitigation, which reduced depression-like behavior in Tat-expressing GT-tg bigenic mice, also could be adopted as an adjunct treatment for HIV. Antioxidant treatment for HIV was proposed more than two decades ago when it was discovered that plasma levels of cysteine and the cysteine dimer cystine, both precursors for intracellular GSH, as well as GSH itself, are depleted in HIV (121). Consistent with this early work, a recent postmortem study reported low frontal cortex GSH levels in HIV+ subjects (122). As oxidative stress increases and antioxidants can suppress HIV virus replication (123–126), antioxidants not only might reduce depression-like behavior but also could help maintain HIV latency. Antioxidants may even counteract undesirable effects of antiretroviral therapy including oxidative stress (127,128), which is higher in patients with better antiretroviral therapy compliance (127).

CONCLUSIONS

Our data demonstrate that HIV-Tat protein can induce depression-like behaviors by increasing oxidative stress. Moreover, our data suggest that treatments capable of mitigating oxidative stress may be effective for reducing depression severity. In light of these findings, care may be necessary when applying HIV reservoir eradication strategies involving oxidative stress-exacerbation (129), which could enhance depression severity. The noninvasive nature of MRS and the improved ability of newly-developed MRS protocols to detect GSH in humans (130,131) may facilitate research in this area, including research into novel treatments for HIV and major depression.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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Figure 1.
(Top panel) Magnetic resonance images of a mouse frontal cortex MRS voxel. (A/P=anterior/posterior; R/L=right/left). **(Bottom panel)** Magnetic resonance spectroscopy (MRS) spectrum from a GT-tg bigenic mouse obtained on day 8 after 7 days of drug treatment. Shown are overall and GABA, glutamate (Glu) and glutathione (GSH) peak fits.

Figure 2.

Tat protein increases immobility time in the tail-suspension test (TST) in a dose-related manner. GT-tg mice (open bars) were treated 7d with saline (N=16, white bar, far left) or Doxycycline (Dox, 25 (N=16), 50 (N=17), or 100 (N=18) mg/kg/d, i.p.) and tested on day 8. Additional GT-tg mice were treated 1d with saline (off white bar, center, N=25) or Dox (100 mg/kg, i.p.; dark grey bar, N=25). Mice lacking the Tat gene (G-tg mice, striped) were treated for 7d with saline (N=21) or Dox (100 mg/kg, i.p.; black striped bar, N=24) and then tested in the TST on day 8. * = $p < 0.05$ vs. matching response of saline-treated (uninduced) GT-tg bigenic mice of same duration. Effect sizes (Cohen's d s) were 0.86 and 1.03 for mice treated with Dox (100 mg/kg/day) for 1 and 7 days, respectively as compared to saline-treated mice.

Figure 3.

Tat protein exposure reduces saccharin, but not water consumption in a two-bottle assay.

Daily consumption (ml/d) of saccharin (**left panel**) or water (**right panel**) shown 6d prior to drug administration, during daily saline (white circles, N=16) or Dox (100 mg/kg/d, i.p.; black squares, N=16) treatment for 7d, and for 5 days thereafter. *= $p < 0.05$ vs. matching response of saline-treated (uninduced) mice of same day. Effect sizes (Cohen's d s) for significantly different paired time points ranged from 1.18 to 1.72.

Figure 4.

Left panel Antioxidant pretreatment mitigates Tat-induced increases in tail suspension test (TST) immobility time. GT-tg mice were treated daily for 7d with saline (white bars, N=30) or Dox (100 mg/kg/d, i.p.; black bars, N=30) alone, or were treated daily with saline (N=20) or Dox (N=22) 20 min after daily pretreatment with methylsulfonylmethane (MSM, 100 mg/kg/d, i.p.; thatched bars). Mice then were tested in the TST 1, 7, 14 and 28 days after drug treatments had concluded. * = $p < 0.05$ vs. matching response of saline-treated (uninduced) GT-tg bigenic mice of same duration; † = $p < 0.05$ vs. matching Dox-treated (Tat-exposed) GT-tg bigenic mice of same duration. Effect sizes (Cohen's d s) for Dox alone vs saline across paired time points ranged from 1.72 (d1) to 0.71 (d28) and effect sizes of MSM plus Dox versus Dox alone ranged from 1.01 (d1) to 1.55 (d28). **Right Panel:** Diethyl maleate (DEM) increases immobility time in the tail-suspension test. GT-tg mice were treated 1-day with saline (N=25, white bars) or Dox (N=25, 100 mg/kg, i.p.; grey bars) alone or pretreated with DEM (34.8 μ M/0.25 ml, i.p.; N=23 (with saline), N=22 (with Dox), thatched bars) and tested the next day. * = $p < 0.05$ vs. matching response of saline-treated (uninduced) GT-tg bigenic mice. Effect sizes (Cohen's d s) versus saline controls were 0.75 (Dox alone), 0.64 (DEM+saline) and 0.84 (DEM+Dox).

Figure 5.

Tat protein induction increases medial frontal cortex (MFC) temperature. GT-tg mice were treated for 7 days with saline (open circles) or Dox (100 mg/kg, i.p.; filled circles) and scanned on day 8. Shown are means $\pm$ SEMs with $N_s=12$ mice per group. Effects of treatment: ANOVA $F_{(1, 22)}=5.42$; $p=0.030$. Effects of partial acquisition order (e.g., time): ANOVA $F_{(4, 88)}=2.65$; $p=0.038$. * $p=0.036$, post-hoc test. First partial acquisition temperature difference was $1.2 \pm 1.1^\circ\text{C}$. Effect size (Cohen's d)=1.11.

Table 1

Medial frontal cortex MRS measures on day 8 in GT-tg mice administered doxycycline or saline (control).

MRS Measure	Sal ^a (Ns)	Dox ^a (Ns)	<i>p</i> -values ^b	(<i>t</i> -statistic, df)
GABA/H ₂ O	15.5 ± 3.4 (14)	14.3 ± 6.9 (14)	0.57	(−0.58, 26)
Gln/H ₂ O	19.9 ± 5.7 (12)	21.1 ± 8.1 (11)	0.68	(0.42, 21)
Glu/H ₂ O	93.3 ± 12.3 (14)	87.0 ± 18.8 (14)	0.30	(−1.06, 26)
Gly/H ₂ O	18.2 ± 6.4 (11)	17.7 ± 9.1 (12)	0.87	(−0.17, 21)
GSH/H ₂ O	15.1 ± 3.5 (14)	19.0 ± 3.3 (14)	0.006	(2.98, 26) ^c
Ins/H ₂ O	45.9 ± 6.7 (15)	47.5 ± 13.9 (14)	0.69	(0.40, 27)
Lac/H ₂ O	13.0 ± 4.6 (13)	12.3 ± 2.6 (11)	0.65	(−0.47, 22)
NAA/H ₂ O	56.0 ± 8.1 (14)	55.7 ± 8.7 (14)	0.93	(−0.09, 26)
Tau/H ₂ O	141.4 ± 17.1 (14)	137.8 ± 25.8 (14)	0.67	(−0.43, 26)
tCho/H ₂ O	17.6 ± 2.8 (15)	16.0 ± 3.8 (14)	0.20	(−1.31, 27)
tCr/H ₂ O	90.8 ± 12.1 (14)	92.5 ± 19.9 (14)	0.78	(0.28, 26)
H ₂ O	230.8 ± 17.8 (15)	236.2 ± 20.8 (14)	0.46	(0.76, 27)

Sal = Saline-treated for 7d; Dox = Dox-treated for 7d; df = degrees of freedom; Gln = glutamine; Glu = glutamate; Gly = glycine; GSH = glutathione; H₂O = unsuppressed water; Ins = myo-inositol; Lac = lactate; NAA = N-acetylaspartate; Tau = Taurine; tCho = total choline; tCr = total creatine;

^a Mean ± SD (N);

^b 2-sided values,

* *p* 0.05;

** *p* 0.01;

^c Effect size (Cohen's *d*)=1.15.

Only measurements with Cramér-Rao Lower Bound (CRLB) values < 30% are included. Measures with Ns < 14 and dfs < 27 indicate that some CRLB values exceeded 30% and/or that some values exceeded two standard deviations from respective group means.