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# Age-related Neuroendocrine, Cognitive, and Behavioral Co-Morbidities Are Promoted by HIV-1 Tat Expression in Male Mice

Alaa Qraeya
*University of Mississippi*, anqrarey@go.olemiss.edu

Fakhri Mahdi
*University of Mississippi*

Marc Kaufman
*Harvard University*

Nicole M. Ashpole
*University of Mississippi*

Jason J. Paris
*University of Mississippi*

# Age-related Neuroendocrine, Cognitive, and Behavioral Co-Morbidities Are Promoted by HIV-1 Tat Expression in Male Mice

Alaa N. Qrareya[1], Fakhri Mahdi[1], Marc Kaufman[3,4], Nicole M. Ashpole[1,2], Jason J. Paris[1,2]

[1]Dept. of BioMolecular Sciences., University of Mississippi [2]Research Institute of Pharmaceutical Science, University of Mississippi

[3]Translational Imaging Center, The McLean Hospital [4]Dept. of Psychiatry, Harvard University

NIH National Institute on Drug Abuse

National Institute of General Medical Sciences

## Background

- Combination antiretroviral therapy (cART) has significantly reduced the incidence of HIV-1 associated dementia and increased life expectancy among infected patients. However, ~50% of the HIV$^+$ population still suffer from neurological and psychological symptoms, including increased incidence of cognitive deficits, anxiety/depression, and neuropathic pain, collectively called neuroHIV [1,3].
- HIV-1 proteins, such as the trans-activator of transcription (Tat), are neurotoxic, remain present in the central nervous system despite combined antiretroviral therapy, and are thought to contribute to neuroHIV [2-5].
- The U.S. HIV$^+$ population is aging with ~60% over the age of 50 years old. These individuals experience accelerated aging and characterized by vulnerability to numerous age-related comorbidities, including endocrine and immune dysfunction, neurocognitive deficits, vascular and metabolic disorders [3-6].
- HIV-1 Tat protein, cART, and chronological aging can disrupt endocrine function, dysregulate the hypothalamic-pituitary-gonadal axis, and impair steroidogenesis. [4-9].

## Hypotheses

**HIV-1 Tat expression will accelerate or accentuate age-related comorbidities including, cognitive, affective, neuromuscular, and neuropathic pain. Tat protein will impact the neuroendocrine capacity concurrent with behavioral deficits.**

## Methods

**Animal Housing**

HIV-1 Tat transgenic mice were generated in the vivarium at the University of Mississippi (University, MS). HIV-1 $Tat_{1-86}$ protein is expressed via a glial fibrillary acidic protein (GFAP)-driven, tetracycline (Tet)-on promoter activated by doxycycline (30 mg/kg, i.p.). Young adult (6-8 months old; $n_{Tat(-)}$ = 9, $n_{Tat(+)}$ = 12) or middle-aged (11-13 months old; $n_{Tat(-)}$ = 12, $n_{Tat(+)}$ = 10) male mice that expressed Tat (Tat+) and their non-Tat-expressing counterparts (Tat-) were housed (3-4/cage) in a temperature- and humidity-controlled room on a reversed 12:12 h light/dark cycle (lights off at 20:00 h).

**Open Filed (OF)**

Mice were placed at the center area of an open field apparatus (40×40×35 cm) and allowed to freely explore for 5 min. Mice were digitally tracked and recorded by ANY-maze software tracking system. A greater proportion of central entries was used as an index for anti-anxiety-like behavior. Total distance travelled was used as an index for locomotor activity.

**Radial Arm Water Maze (RAWM)**

Mice were placed in an 8-arm radial arm water maze with the goal of reaching a hidden platform. During training, mice performed 6 trials/day with each trial ending when the mouse either reached the hidden platform or after 60 s. When mice failed to locate the platform, they were gently guided to it and allowed to remain for 15 s. The total number of errors and greater latency to reach the platform were used as indictor of a hippocampus-dependent learning and memory deficits.

**Thermal Hyperalgesia**

A radiant heat source was applied to the middle plantar surface of the hind paw (2.5 ℃/sec) until paw withdrawal. Each mouse underwent 4 trials (2: right and 2: left) with a 3 -5 min interval break between trials.

**Steroid Extraction/ Enzyme-Linked Immunosorbent Assay (ELISA)**

Steroids were extracted from serum via ether-snap freezing. Samples were reconstituted in extraction buffer and assessed via ELISA for corticosterone, estradiol, and testosterone per manufacturer instructions (Neogen Life Sciences, Lexington, KY). Plates were read on a CLARIOstar microplate reader (BMG Labtech Inc., Cary,NC) at 650 nm absorbance.

**Ultra Performance Liquid Chromatography (UPLC) - Mass Spectrometry (MS)**

Brain regions (prefrontal cortex, midbrain, hippocampus) were grossly-dissected. Charcoal-stripped brain tissues derived from Tat-tg mice were used for calibration and quality control. Steroid extraction as achieved via protein precipitation with samples homogenized in 100 µl of PBS (pH 7.4). Samples were precipitated with 100 µl of acetonitrile followed by vortexing for 2 min and centrifugation. Following centrifugation, supernatants were mixed with 50 µl of derivatizing solution and incubated for 1 h. 20 µl of the internal standard solution (1 µg/ml) was added and vortexed. For analysis, 2 µl of sample was injected onto the UPLC-MS/MS instrument.

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## Advanced Age and Tat Exposure Altered Steroidogenesis in Male Mice

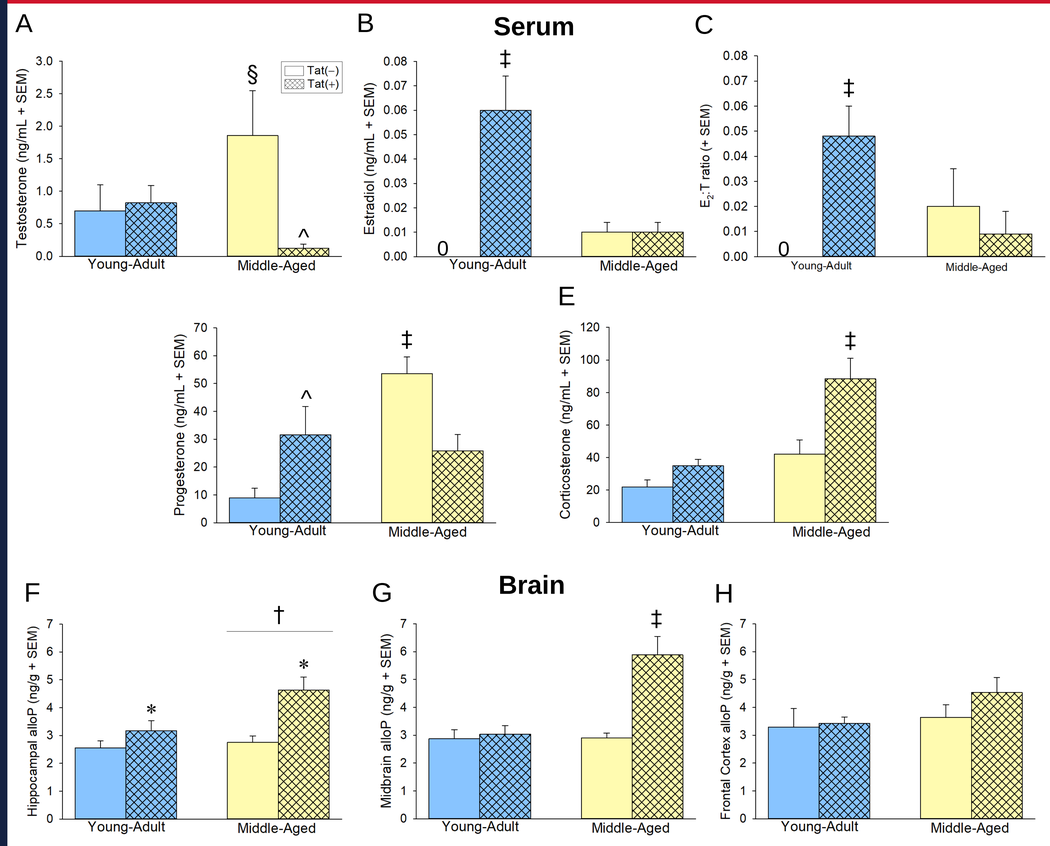


Figure1. Circulating (serum; ng/mL) and central (ng/g) steroid hormones among young adult and middle-aged HIV-1 Tat-transgenic male mice [Tat(+)] or their non-Tat–expressing age-matched counterparts [Tat(-)]. (A) Testosterone (T), (B) estradiol ($E_2$), (C) the $E_2$:T ratio, (D) progesterone, and (E) corticosterone detected in serum. (F) Hippocampal, (G) midbrain, and (H) frontal cortex allopregnanolone (alloP) detected in grossly-dissected brain regions. ∗ main effect for Tat(+) mice to differ from Tat(−) controls. † main effect for middle-aged mice to differ from young adults. ^ significant interaction wherein indicated group differs from respective Tat(−) controls. ‡ indicated group differs from all other groups. § indicated group differs from young adult Tat(−) controls (two-way ANOVA, $p < 0.05$)

## Advanced Age or Tat Reduced Cognitive Performance in Male Mice

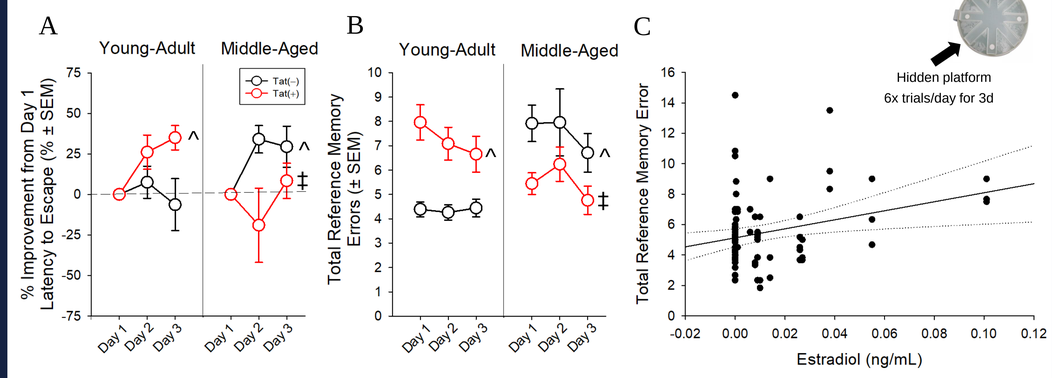


Figure 2. (A-B) Spatial memory performance in a radial arm water maze and (C) simple linear regressions for circulating and central steroid hormones among young adult and middle-aged HIV-1 Tat-transgenic male mice [Tat(+)] or their non-Tat-expressing age-matched counterparts [Tat(−)]. (A) Proportion of mice that exhibited improvement from day one performance (latency to escape). (B) Total frequency of errors. (C) circulating estradiol and frequency of errors. ∗ main effect for Tat(+) mice to differ from Tat(−) mice. ^ interaction effect wherein indicated group differs from young adult Tat(−) controls. ‡ indicated middle-aged Tat(+) groups differs from middle-aged Tat(−) and young adult Tat(+) mice. Regression lines (solid) are depicted with 95% confidence intervals (dotted), (repeated measure ANOVA, $p < 0.05$)

## Advanced Age or Tat Increased Anxiety-Like Behavior Among Male Mice

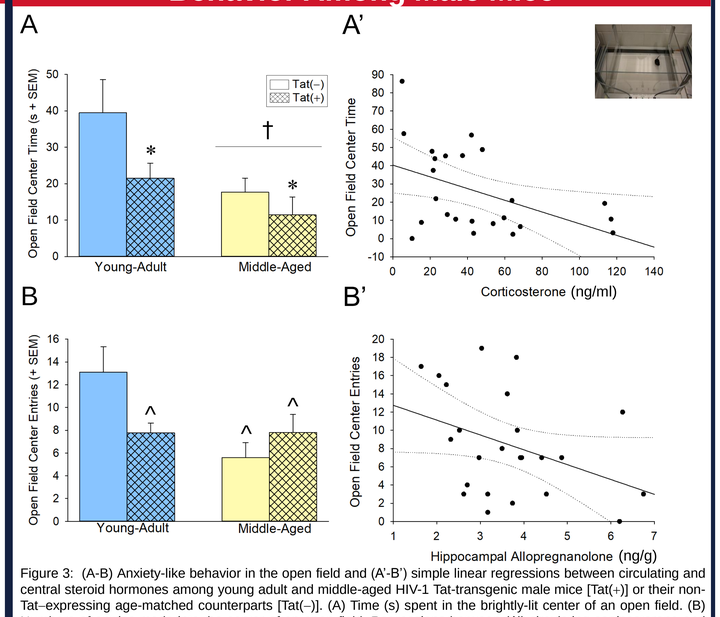


Figure 3: (A-B) Anxiety-like behavior in the open field and (A'-B') simple linear regressions between circulating and central steroid hormones among young adult and middle-aged HIV-1 Tat-transgenic male mice [Tat(+)] or their non-Tat–expressing age-matched counterparts [Tat(−)]. (A) Time (s) spent in the brightly-lit center of an open field. (B) Numbers of entries made into the center of an open field. Regressions between (A') circulating corticosterone and center time, (B') hippocampal allopregnanolone and center entries.∗ main effect for Tat genotype wherein Tat(+) mice to differ from Tat(−) mice. † main effect for middle-aged mice to differ from young adult mice. ^ significant interaction wherein indicated group differs from young adult Tat(-) controls. Regression lines (solid) are depicted with 95% confidence intervals (dotted), (two-way ANOVA, $p < 0.05$)

## Advanced Age and Tat Reduced Neuromuscular Strength Among Male Mice

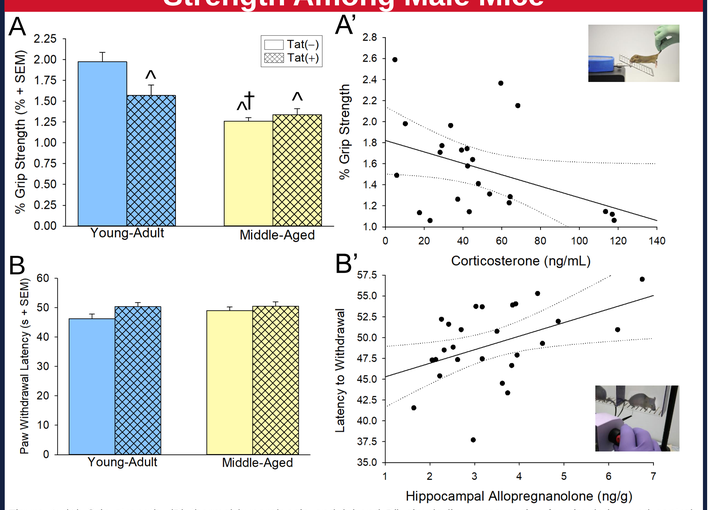


Figure 4: (A) Grip strength, (B) thermal hyperalgesia and (A' and B') simple linear regression for circulating and central steroid hormones among young adult and middle-aged HIV-1 Tat-transgenic male mice [Tat(+)] or their non-Tat-expressing age-matched counterparts [Tat(−)]. (A) Grip strength threshold forelimbs and hindlimbs together . (B) Paw-withdrawal latency (s) in a thermal probe test. Simple linear regressions between (A') circulating corticosterone and forelimbs and hindlimbs together (B') hippocampal allopregnanolone and paw-withdrawal latency. ∗ main effect for Tat(+) mice to differ from Tat(−) mice. † main effect for middle-aged mice to differ from young adults. ^ significant interaction wherein indicated group differs from young adult Tat(−) controls. Regression lines (solid) are depicted with 95% confidence intervals (dotted), (two-way ANOVA, $p \leq 0.05$)

## Conclusion

*Thus, HIV-1 Tat promoted cognitive deficits, anxiety–like behavior, and neuromuscular middle-aged male mice in a manner coincident with their neuroendocrine aging status. Future studies will determine whether administration of exogenous steroid hormones to aged Tat-exposed exert cognitive, affective, and nociceptive benefits.*

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