HIV-1 Tat Promotes Age-Related Cognitive, Anxiety-like, and Antinociceptive Impairments in Mice that are Moderated by Aging Endocrine Status

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

[1]Dept. of BioMolecular Sciences Dept., 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, 4].
- 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 [4-6].
- HIV-1 Tat protein, cART, and chronological aging can disrupt endocrine function, dysregulate the hypothalamic-pituitary-gonadal axis, and impair steroidogenesis. [4-7].

Hypotheses

HIV-1 Tat expression will accelerate aging as assessed by cognitive, affective, and antinociceptive comorbidities. Tat will interact with neuroendocrine aging such that maintenance of reproductive status will be associated with reduced comorbidity.

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.). Aged (15-19 mos), male (n=26) and female (n=48) mice that expressed Tat (Tat+) and their non-Tat-expressing counterparts (Tat-) were housed (4-5/cage) in a temperature- and humidity-controlled room on a reversed 12:12 h light/dark cycle (lights off at 20:00 h).

Vaginal Cytology

Vaginal lavage was used to collect epithelial cells to determine estrous cycle phase. The morphology of cells and/or the presence or absence of leukocytes were used to identify estrous stages; proestrus (characterized by a predominance of nucleated cells), estrous (characterized predominantly by cornified cells), metestrus (characterized by nucleated, cornified, leukocytic cells), and diestrous (characterized by a majority presence of leukocytes).

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.

Elevated Plus Maze (EPM)

Mice were placed in the center of an elevated plus maxe (5× 5 cm) and allowed to explore the maze for 5 min. Animals were tracked digitally using Noldus Ethovision tracking software. The latency to enter, and time spent on, the open arms of the maze were considered an index of anti-anxiety-like behavior.

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. 8 days after acquisition, memory was assessed in consecutive reversal probe and reversal learning trials. The total number of errors and greater latency to reach the platform were used as indictor of a hippocampus-dependent learning and memory deficits.

Electronic Von Frey (eVF) Mechanical Allodynia

The eVF probe was applied to the middle plantar surface of the hind paw and force was incrementally increased until paw withdrawal. The required force (g) to produce the withdrawal was recorded. Each mouse was tested in eight trials (4: right and 4: left) alternating between right and left paws with a 3-min interval break between trials.

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.

Tat Exposure in Aged Males and Post-Estropausal Females Increases Memory Errors and Latency to Escape

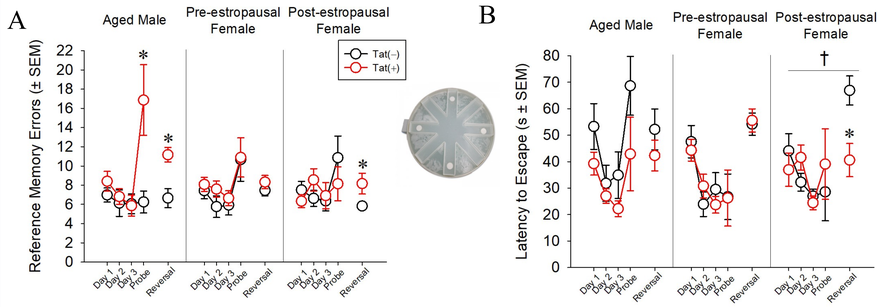

Figure 1: (A) Tat expression increased reference memory errors in males and post-estropausal females. (B) Post-estropausal females had a greater latency to escape compared to pre-estropausal females. * indicates Tat+ significantly differs from respective Tat- control group. † indicates significant difference from pre-estropausal group (repeated measures ANOVA, $p < 0.05$).

HIV-1 Tat Expression or Post-Estropausal Status Exacerbates Anxiety-Like Behavior in an Open Field and Elevated Plus Maze

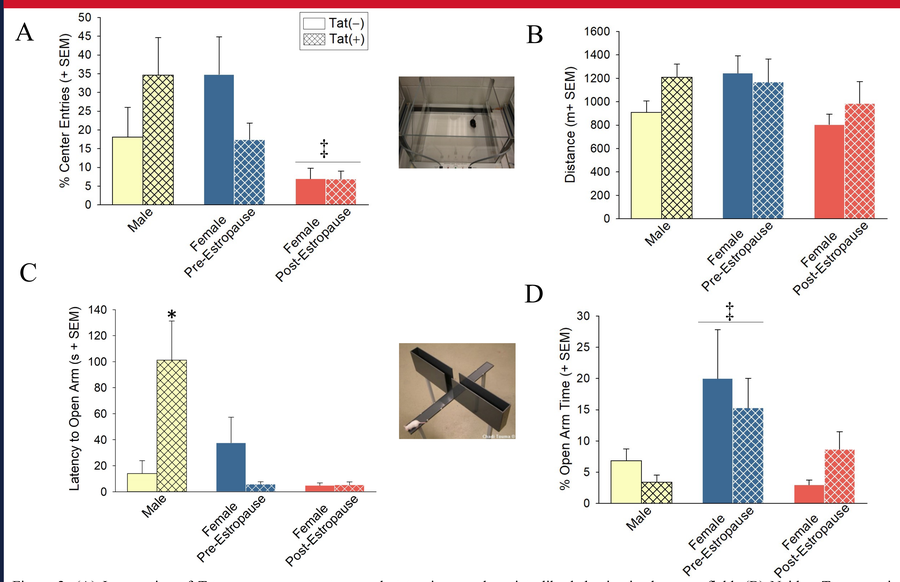

Figure 2: (A) Irrespective of Tat exposure, post-estropausal status increased anxiety-like behavior in the open field. (B) Neither Tat nor aging influenced distance traveled in the open field. (C) Tat+ males had a greater latency to enter the open arm of an elevated plus maze. (D) Pre-estropausal females demonstrated decreased anxiety–like behavior in the elevated plus maze compared to all other groups. * indicates Tat+ significantly differs from respective Tat- control group. ‡ indicates significant difference from all other groups (2-way ANOVA, $p < 0.05$).

HIV-1 Tat Expression, in Aged Mice, Increases Mechanical Allodynia. Neither Tat nor Aging Impair Thermal Hyperalgesia

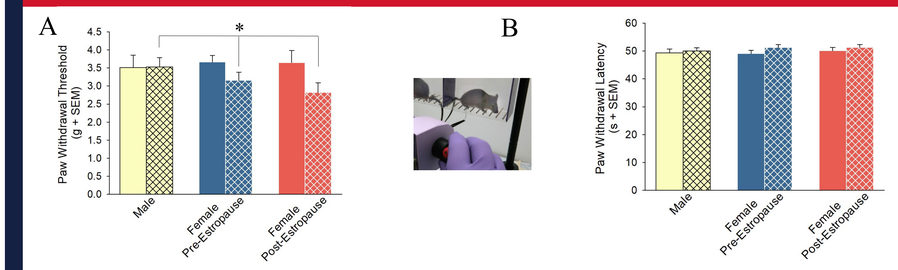

Figure 3: (A) There was a main effect for Tat expression to increase mechanical allodynia. (B) Neither Tat nor endocrine status influenced thermal hyperalgesia. * indicates Tat+ significantly differs from respective Tat- control group (2-way ANOVA , $p < 0.05$).

HIV-1 Tat Contributes to Disrupt Endocrine System

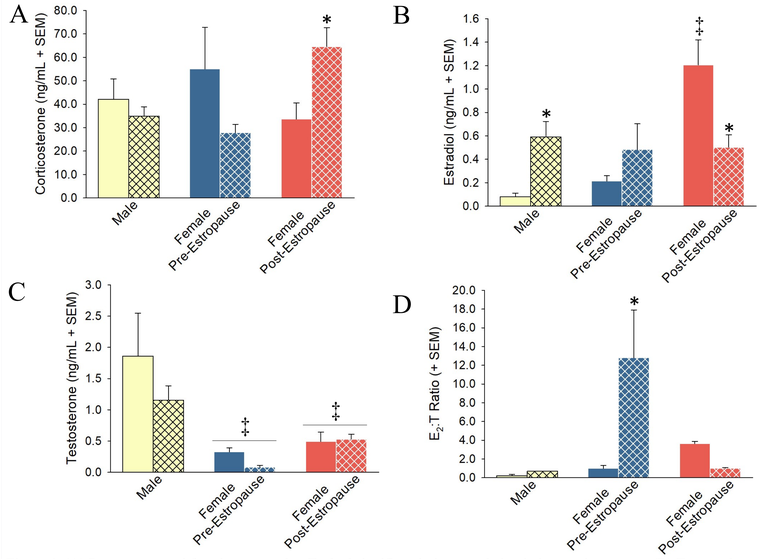

Figure 4: (A) Post-estropausal females exposed to Tat had significantly increased circulating corticosterone. (B) Tat+ males and post-estropausal females had significantly less circulating estradiol (E_2). (C) Males had more circulating testosterone (T) than females. (D) The E_2:T ratio increased in Tat+, pre-estropausal females. * Tat+ significantly differs from Tat- control. † significant difference from pre-estropausal group. ‡ significant difference from all other groups (2-way ANOVA, $p < 0.05$).

Summary

- HIV-1 Tat expression and aging significantly impaired cognition. Among males, those exposed to Tat made more errors. Post-estropausal females had longer latencies to find the platform than did their pre-estropausal counterparts and made more errors when Tat-exposed **(Fig. 1A-B).**
- HIV-1 Tat expression increased anxiety-like behavior among males (**Fig. 2C**) and transition to post-estropause increased anxiety-like behavior in an open field **(Fig. 2A)** and elevated plus maze **(Fig. 2D)**.
- Tat increased mechanical allodynia with no effect on thermal hyperalgesia **(Fig. 3A-B).**
- In post-estropause, Tat increased corticosterone and decreased estradiol. Whereas, Tat-exposed males had increased estradiol. The E:T ratio was increased in Tat-exposed pre-estropausal females **(Fig. 4A-D).**

Conclusion

Thus, HIV-1 Tat promoted cognitive deficits, anxiety–like behavior, and allodynia among aged mice in a manner coincident with their neuroendocrine aging status. Aged females that maintained their reproductive capacity may be more resilient to Tat's accelerating effects on age. Future studies will determine whether administration of exogenous steroid hormones to aged Tat-exposed exert cognitive, affective, and nociceptive benefits.

References

1. Cañizares, Silvia, Mariana Cherner, and and Ronald J. Ellis. 2014. *Seminars in Neurology* 34 (1): 27–34.
2. Ensoli, Barbara, Valeria Fiorelli, Fabrizio Ensoli, Aurelio Cafaro, Fausto Titti, Stefano Buttò, Paolo Monini, Mauro Magnani, Antonella Caputo, and Enrico Garaci. 2006. *Aids* 20 (18): 2245–61. Aziz-Donnelly, Angela, and Taylor B. Harrison. 2017. *Current Treatment Options in Neurology* 19 (10).
3. Cherner, Mariana, Ronald J. Ellis, Deborah Lazzaretto, Corinna Young, Monica Rivera Mindt, J. Hampton Atkinson, Igor Grant, and Robert K. Heaton. 2004. *Aids* 18 (SUPPL. 1).
4. Slama, Laurence, Lisa P. Jacobson, Xiuhong Li, Frank J. Palella Jr, Joseph B Margolick, Lawrence A. Kingsley, Dorothy J. Wiley, et al. 2016. *The Journal of Acquired Immune Deficiency Syndromes*.
5. Imai, Kentaro, Madeline Y. Sutton, Rennatus Mdodo, and Carlos del Rio. 2013. *Obstetrics and Gynecology International* 2013: 1–11.
6. Maingat, Ferdinand G., Maria J. Polyak, Amber M. Paul, Pornpun Vivithanaporn, Farshid Noorbakhsh, Samir Ahboucha, Glen B. Baker, Keir Pearson, and Christopher Power. 2013. *FASEB Journal*27 (2): 725–37.

Acknowledgments:

This work was supported by funds from NIDA (R00 DA039791), NIGMS (Adm. Suppl. to P30GM122733), Office of the Director (S10 OD026751), and The University of Mississippi