HIV-1 Tat Dysregulates the Hypothalamic-Pituitary-Adrenal Stress Axis and Potentiates Oxycodone-mediated Psychomotor and Anxiety-like Behavior of Male Mice

Mohammed Salahuddin[1], Fakhri Mahdi[1], Jason J. Paris[1,2]

[1]Department of BioMolecular Sciences and [2]The Research Institute of Pharmaceutical Sciences, University of Mississippi, University, MS, USA

NIH National Institute on Drug Abuse

Abstract

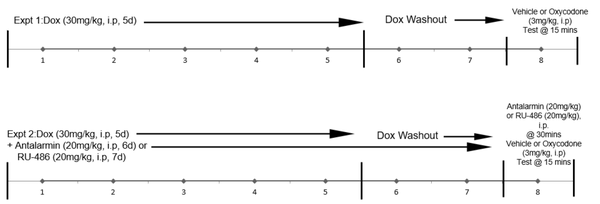
Human immunodeficiency virus (HIV) is associated with co-morbid affective and stress-sensitive neuropsychiatric disorders (collectively referred to as neuroHIV) that afflict ~50% of infected individuals. One factor that may contribute to neuropathology is the HIV-1 regulatory protein, trans-activator of transcription (Tat), which promotes anxiety-like behavior that may be exacerbated by opioid use/abuse. Our prior findings show that conditional expression of HIV-1 Tat in transgenic mice interacts with acute oxycodone administration to activate the hypothalamic-pituitary-adrenal (HPA) stress axis, concurrent with psychomotor/affective dysfunction. Tat-mediated effects on opioid responding may facilitate vulnerability to stress-related disorders that impact substance use. We hypothesized that HPA dysregulation may contribute to HIV-1 Tat-mediated interactions with oxycodone. When administered acutely, oxycodone (3 mg/kg) increased psychomotor behavior in an open field and these effects were greater in transgenic mice that conditionally-expressed the HIV-1 Tat protein [Tat(+)] compared to their control counterparts [Tat(-)]. Similar to observations in HIV$^+$ patients, Tat(+) mice demonstrated greater circulating corticosterone than did Tat(-) controls at baseline. However, Tat(+) mice mounted an insufficient corticosterone response to stress when exposed to oxycodone challenge. Furthermore, Tat expression and/or oxycodone administration produced increased anxiety-like effects in a light-dark transition task. The CRF1 receptor antagonist, Antalarmin, and GR receptor antagonist, RU-486 attenuated Tat's capacity to potentiate oxycodone psychomotor effects. Thus, actions of Tat protein may underlie HIV-1 mediated HPA dysfunction and the incapacity to mount an appropriate stress response may influence the effects of clinical opioids on neuroHIV-related behavior.

Hypotheses

- ***In vivo*, HIV-1 Tat and oxycodone will interact to potentiate psychomotor and anxiety-like behavior involving hypothalamic-pituitary-adrenal (HPA) axis activation.**
- **Antalarmin and/or RU-486 may attenuate combined Tat and oxycodone psychomotor behavior.**

Methods

Animal Subjects: Transgenic mice were bred in the vivarium at the University of Mississippi (University, MS). Tat (+) mice expressed a Tat $_{1\text{-}86}$ protein that became transcriptionally-active in the presence of doxycycline (induced via doxycycline injection, 30mg/kg/d for 5d). Tat (-) mice expressed on the transcription factor necessary to activate transgene induction, but did not express the transgene itself. Anxiogenic effects of Tat induction have been previously-observed using these mice . Mice were kept in a temperature- and humidity-controlled environment on a 12:12 h light:dark cycle (lights off at 09:00 h) with *ad lithium* access to food and water.



Behavioral Assessment: Mice were behaviorally-tested in open field and light dark transition task. All tests were completed within 8 days of doxycycline induction and occurred 2-3 h into the dark phase of the light cycle. Data were encoded by an ANY-maze behavioral tracking system (Stoelting Co., Wood Dale, IL).

Chemicals: Tat $_{1\text{-}86}$ was induced in transgenic mice [Tat (+) or Tat (-)] via doxycycline injection (30 mg/kg, i.p.; Cayman Chemical, Ann Arbor, MI). Antalarmin (20 mg/kg, i.p.; 7 days Cayman Chemical, Ann Arbor, MI) and/or Mifepristone (20 mg/kg, i.p.; 8 days Cayman Chemical, Ann Arbor, MI) Oxycodone was diluted to concentration in sterile saline (0.9%) and administered once (3 mg/kg, i.p.) 15 min prior to testing..

Enzyme-linked immunosorbent assay (ELISA): Circulating corticosterone was assessed via ELISA kit per manufacturer instructions (Neogen Life Sciences). Plates were read on a CLARIOstar microplate reader (BMG Labtech Inc., Cary, NC).

HIV-1 Tat and/or Oxycodone potentiated psychomotor and anxiety-like behavior and produced adrenal insufficiency

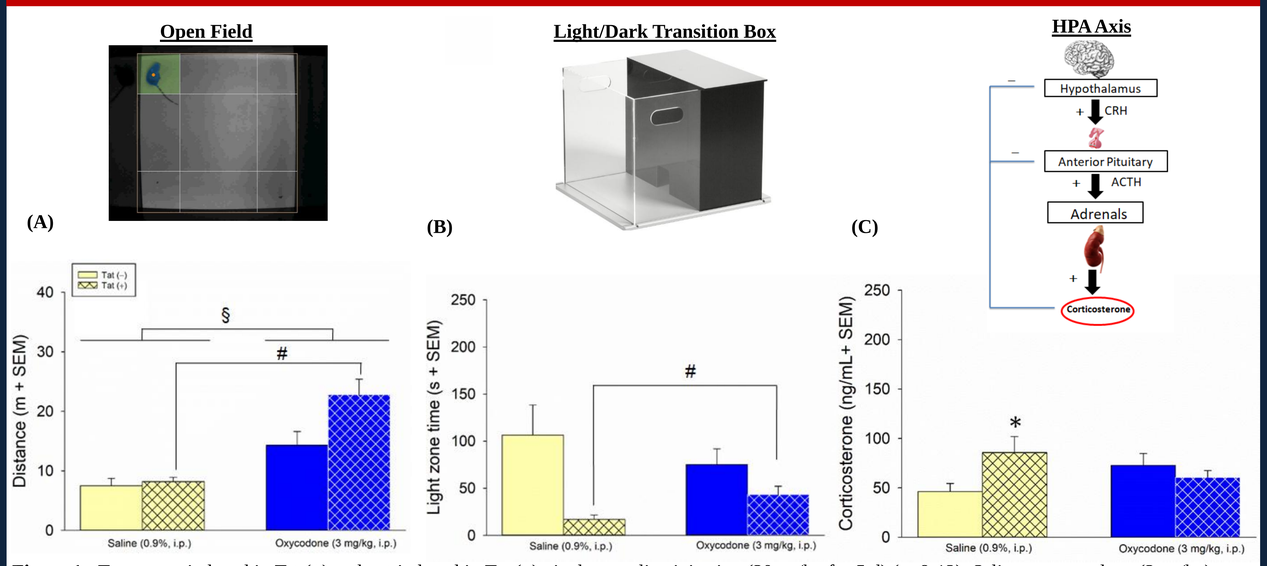


Figure 1: Tat$_{1\text{-}86}$ was induced in Tat (+) and not induced in Tat (–) via doxycycline injection (30mg/kg for 5 d) (n=8-12). Saline or oxycodone (3mg/kg) were administered 15 minutes prior to behavior testing and assessed in an open field (n = 8-12) and a light/dark transition task (n=7-12);**(A)** Distance (m) traveled in an open field. **(B)** The time spent in light chamber of light-dark transition task; **(C)** Circulating corticosterone (ng/mL; n=8-10). # indicates a main effect of genotype wherein Tat (+) mice differ from Tat (–) controls **in panel A and B.** * indicates interaction wherein saline administered Tat (+) mice differ from respective Tat (–) controls **in panel C**; § indicates a main effect for oxycodone to differ from saline administration **in panel A**; $p \leq 0.05$.

Antalarmin and/or RU-486 attenuated combined Tat and oxycodone mediated psychomotor behavior, and influenced circulating steroids

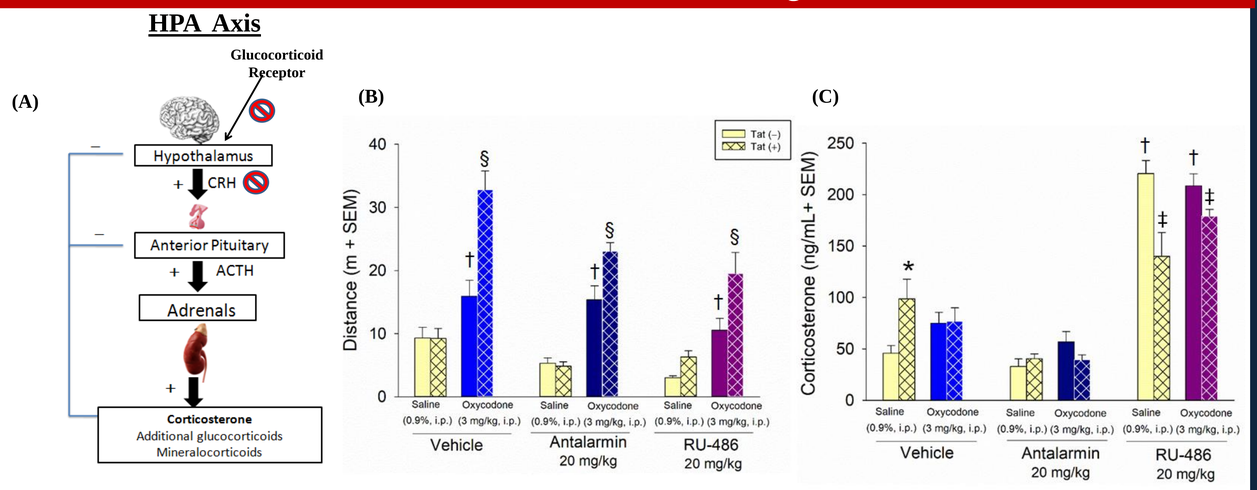


Figure 2: **(A)** The HPA axis; **(B)** Distance (m) traveled in an open field among Tat (–) and Tat (+) mice acutely-administered saline (0.9%) or oxycodone 3 mg/kg; n=8-9). **(C)** Circulating corticosterone (ng/mL; n=8-9). § indicates an interaction wherein oxycodone-administered Tat (+) mice **in panel B** differ from all other groups; † indicates an interaction wherein oxycodone-administered Tat (–) mice **in panel B** differ from all other groups. * indicates an interaction wherein saline-administered Tat (+) mice differ from respective Tat (–) controls in **panel C.** † indicates a 3-way interaction wherein the Tat (–) mice differs from all other groups, except from each other in **panel C**, ‡ indicates a 3-way interaction wherein Tat (+) mice differs from all other groups, except its respective Tat (–) control, $p \leq 0.05$

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Summary

- Tat expression and/or oxycodone greatly potentiated psychostimulatory effects compared to respective controls (**Fig. 1A**).
- Tat caused an increase in anxiety-like behavior in mice, with significantly less time spent in light zone (**Fig. 1B**).
- Tat increased circulating corticosterone than did Tat(-) controls at baseline, however Tat (+) mounted an insufficient corticosterone response to oxycodone challenge indicative of adrenal insufficiency (**Fig. 1C**).
- Antalarmin and/or RU-486 attenuated combined Tat and Oxycodone psychomotor behavior (**Fig. 2B**).
- RU-486 potentiated circulating CORT levels, but Tat and/or oxycodone exposure failed to produce higher levels indicative of secondary adrenal insufficiency (**Fig. 2C**).

Conclusions

HIV and clinical opioids can interact to activate stress pathways, influencing psychomotor and affective behavior which may be correlated with HPA axis dysregulation. Antalarmin and RU-486 were able to attenuate the psychomotor behavior indicating the involvement of CRF and GR receptor as potential targets for neuroHIV behavior in HIV infected population.

References

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