University of Mississippi

eGrove

Spring 5-11-2020

HIV-1 TAT Interactions with Opioids are Modulated by Progesterone and Estradiol

Dejun Jackson
University of Mississippi

Follow this and additional works at: https://egrove.olemiss.edu/hon_thesis

Part of the Behavioral Neurobiology Commons, Molecular and Cellular Neuroscience Commons, and the Pharmacology Commons

HIV-1 TAT INTERACTIONS WITH OPIOIDS ARE MODULATED BY
PROGESTERONE AND ESTRADIOL

By

Dejun Corterio Jackson

A thesis submitted to the faculty of The University of Mississippi in partial fulfillment of
the requirements of the Sally McDonnell Barksdale Honors College

Oxford

April 2020

Approved by

_____

Advisor: Jason Paris, Ph.D.

_____

Reader: Alberto Jose Del Arco Gonzalez,
Ph.D.

_____

Reader: Dale G. Nagle, Ph.D.

ACKNOWLEDGEMENTS

ABSTRACT

HIV-1 Tat interactions with opioids are modulated by progesterone and estradiol

(Under the direction of Dr. Jason Paris)

HIV infection and combined substance abuse are comorbid epidemics. Previous studies show that concurrent opioid drug use may potentiate HIV-1-mediated neurotoxicity partly via interactions with opioids. Preclinical studies suggest that the HIV-1 trans-activator of transcription (Tat), an HIV regulatory protein, can synergize with opioids to exacerbate its already neurotoxic effects. However, its interactions with clinical opioids, such as oxycodone, have yet to be elucidated. Additionally, Tat disrupts a number of systems including the dopaminergic system, which contribute to its capacity to potentiate the rewarding effects of abused drugs. Although the neurotoxic effects of Tat may be inhibited by gonadal steroids *in vitro*, such as estradiol (E_2) and progesterone (P_4), preclinical work suggests that estradiol may also potentiate drug reward/reinforcement as well. Little is known about the behavioral interactions between Tat and E_2. In a 2-bottle choice task, we found that Tat expression in mice significantly increased morphine preference on the first day of morphine presentation. E_2 (0.09 mg/kg, s.c., QOD) significantly increased morphine consumption, irrespective of Tat expression. Following naloxone-precipitated withdrawal, Tat or E_2 increased jumping behavior. In complementary in vitro experiments, Tat increased cell death in SH-SY5Y human neuroblastoma cells and oxycodone did not further potentiate this effect. Irrespective of whether cells were treated with Tat alone or in combination with oxycodone, any concentration of E_2 (1 or 10 nM), or the highest concentration of P_4 (100 nM, but not 10 nM) significantly attenuated Tat-mediated cell death. These data suggest that E_2 may display parallel protective qualities over Tat's

capacity to potentiate withdrawal and promote cell death. However, caution is warranted given E_2's capacity to increase opioid self-administration. These data lend further evidence to support the potential neuroprotective efficacy of gonadal steroid hormone-based therapeutics and further our understanding of their interactions with HIV-1 and the clinically-prescribed opioid, oxycodone.

TABLE OF CONTENTS

LIST OF FIGURES

1. **Introduction**

1.1. HIV-1 Patients and Neurological Dysfunction

With the introduction of combined antiretroviral therapy (cART), HIV type 1 (HIV-1) has transitioned to a chronic disease (Nakagawa et al., 2013). These therapies prove efficacious in reducing viral load and promoting the recovery of the immune system, thus increasing the life expectancy of patients with HIV-1 (Li et al., 1998). However, due to cART's poor retention within the central nervous system (CNS) and inability to target latent viral reservoirs, HIV-1 proteins continue to be produced. Some HIV-1 proteins that are produced from these viral reservoirs are neurotoxic and promote a constellation of neurological disorders including affective, cognitive, and motor dysfunctions, collectively termed neuroHIV. Despite the success of cART, approximately 50% of individuals with HIV-1 suffer from neuroHIV (Tozzi et al., 2007; Simioni et al., 2010). As such, it is important to elucidate the underlying causes and mechanisms of neuroHIV, to better understand and treat this phenomenon.

1.2. The Trans-activator of Transcription: An Excitotoxic HIV-1 Protein

Among the soluble HIV-1 proteins that accumulate within the CNS, the HIV-1 trans-activator of transcription (Tat) is among the most toxic, and plays a critical role in the virus' propagation in the CNS. HIV-1 Tat is critical in the replication of the virus (Jeang et al., 1991). Furthermore, findings from *in vitro* studies suggest that Tat administration is linked to increased neuronal apoptosis, leading to cellular damage and death (Kruman et al., 1998). Studies also suggest that HIV-1 Tat neurotoxicity is likely mediated by the protein's ability to alter calcium homeostasis (Haughey et al. 1999; Haughey and Mattson,

2002; Hu, 2016), stimulate glutamate receptors to cause excitotoxic effects (Gras et al., 2003), and induce oxidative stress (Kruman et al., 1998; Perl and Banki, 2000), which is a consistent biomarker found in patients with HIV associated dementia (Sacktor et al., 2004). Moreover, Tat is also suspected to play a role in the synaptodendritic injury that is observed in HIV-1 patients (Bertrand et al., 2013) which contributes to neurocognitive deficits. As such, various mechanisms underlie Tat-mediated neuronal dysfunction that likely contributes to the neuropathology of neuroHIV.

1.3. Neurologic Effects of HIV-1 Tat and Combined Substance Use

HIV-1 infection and combined substance use have become increasingly co-morbid (Gorman, 1998; Mathers et al., 2008), and a substantial amount of evidence suggest concurrent HIV-1 infection and combined substance use can increase the prevalence and severity of neuroHIV among infected patients (Bell et al., 2006). A number of studies also suggest that the HIV virus' infiltration and subsequent damage and dysregulation of structures in dopamine system, a well-known mediator of drug reward and reinforcement (Kalivas & Volkow, 2005; Koob & Volkow, 2016), may be a contributing factor in the development and worsening of neuroHIV (Bell et al., 2006; Gaskill et al., 2013). Interestingly, HIV-1 Tat has been shown to be involved in HIV mediated alterations in the dopamine system (Fitting et al., 2015). More specifically, *in vitro* studies show that Tat's involvement includes dysregulation of dopamine transporters, and interaction with dopamine receptors to cause neurotoxicity (Bucci, 2015; Kesby et al., 2018; Midde et al., 2012; Silvers et al., 2007). Furthermore, addiction studies also suggest that Tat may interact with psychostimulant drugs of abuse to potentiate their effects. When modeled in animals, drug self-administration, a key tenet of the addiction cycle, may be enhanced by the

presence of Tat. For instance, HIV-1 Tat expression has been shown to potentially increase the rewarding effects of cocaine in male mice (Paris et al, 2014). Another study suggests that Tat expression can lead to reward deficits, but increase sensitivity to methamphetamine related reward enhancements (Kesby et al., 2016). In another study, HIV-1 Tat mice also displayed increased morphine-conditioned place preference (Gonek et al., 2018).

1.4. Sex Differences in HIV-1 and Substance Abuse-Related Neurological Sequelae

A growing amount of evidence suggests that there is also likely sex difference in the vulnerability to neurological dysfunction associated with HIV. In the general population, women tend to be diagnosed with anxiety and depressive disorders more often than men (McLean et al., 2011). Interestingly, this sex difference is reversed with some studies suggesting that women with HIV may exhibit a decreased vulnerability to HIV-associated affective disorders (Bing et al., 2001; Lopes et al., 2012) as well as reduced neurological dysfunction (Cabrera-Muñoz et al., 2012); though, these findings are not always observed. Some studies report a greater vulnerability among women or do not find a gender difference (Sewell et al., 2000; Tsao et al., 2004). Some of these effects are recapitulated in animal models. When exposed to Tat in an *in vivo* setting, female mice exhibit less neuronal degeneration and behavioral deficits than do males (Hahn et al., 2015). Past observations suggest that sex steroid hormones may play a role. Ovariectomized, Tat expressing, female mice exhibit a decrease in anxiety-like behavior when administered progesterone (Paris et al., 2014; 2016).

When combined with comorbid substance use, these HIV-1 related neurological effects can be aggravated. Various studies suggest that there are significant differences in addiction-related behavior among females and males. However, the literature on addiction

has been mainly comprised of research on male models. Studies show that a greater proportion of men engage in substance abuse compared to women (Becker et al., 2017). On the other hand, women tend to escalate use of drugs and alcohol to the point of addiction faster than men (Bobzean et al., 2014). This is also true in animal models, as female rats acquire cocaine self-administration behaviors faster than male rats (Becker, 2016; Becker & Koob 2016). Some studies speculate that sex differences in the addiction cycles may be mediated by sex hormones, such as estradiol and progesterone.

In vivo experiments suggest that estradiol promotes cocaine self-administration in rats, while progesterone decreased self-administration (Larson et al., 2007). In female ovariectomized rats, estradiol treatment increases drug-taking behaviors and behavioral responses to cocaine and amphetamine (Peris et al., 1991; Hu & Becker, 2003). Estradiol's effect is thought to be mediated through its ability to alter dopaminergic functioning. For example, some studies found that estradiol exposure can increase dopamine release in the striatum (Becker, 1990) within the mesolimbic dopaminergic pathway. Coincidingly, psychostimulant induced increases of dopamine were enhanced in ovariectomized female rats when treated with estradiol compared to estradiol treated male rats (Cummings et al., 2014). However, *in vitro* studies suggest that estradiol displays neuroprotective qualities against HIV-1 Tat (Kendall et al., 2005; Salahuddin et al., 2019; Turchan et al., 2001; Wallace et al., 2006). The behavioral effects of estradiol on opioid reward and reinforcement in combination with HIV-1 Tat expression have yet to be elucidated.

1.5. Opioid use and HIV infection

With opioid drug abuse and opioid prescriptions on the rise in the HIV population (Williams & Bisaga 2016; Dutta & Roy 2012; Edelman et al., 2012), it is becoming more pertinent to understand the interactions between the opioids and HIV-1. Studies suggest that opioid abuse exacerbates the effects of neuroHIV (Anthony et al., 2008). However, the current literature is discrepant. Some studies suggest that morphine, the prototypical opiate, may display protective qualities by slowing AIDS progression in non-human primate models (Donahoe et al., 2009). Other studies suggest that morphine stimulates replication of viral HIV-1 proteins (Schweitzer et al., 1991), and also interacts with HIV-1 Tat to potentiate its neurotoxic effects *in vitro.* Furthermore, evidence suggests that opioid abuse increases the synaptodendritic degeneration when opioids and HIV-1 Tat are administered in conjunction in mice (Fitting et al., 2010a; Fitting et al., 2014). Interestingly, oxycodone's interaction with HIV-1 proteins has remained under-investigated and largely unknown. This is especially clinically concerning as HIV patients have an increased probability of suffering from chronic pain (Marcus et al., 2000), and these opioid analgesic prescriptions are increasing in this patient population (Miaskowski et al., 2016; Merlin et al., 2016).

In experiment 1 of the present study, we aimed to investigate the *in vivo* effects of estradiol on HIV-1 Tat-mediated morphine reinforcement. To examine this, we administered estradiol on a QOD (every other day) regimen and used the two-bottle choice paradigm to assess morphine self-administration in ovariectomized female mice in experiment 1. To assess morphine dependence, we precipitated withdrawal with the nonselective opioid receptor antagonist, naloxone, in experiment 2. Shortly after naloxone administration, we observed and quantified displays of withdrawal behaviors. In

experiment 1, we hypothesized that estradiol would potentiate HIV-1 Tat mediated morphine reinforcement and later increase withdrawal behaviors in Experiment 2. In experiment 3, we conducted a live/dead assay using differentiated SH-SY5Y human neuroblastoma cells to assess whether oxycodone could potentiate the neurotoxic effects of HIV-1 Tat in the absence of glial inputs. Furthermore, we investigated whether physiological concentrations of estradiol (E_2) and progesterone (P_4) could attenuate Tat-mediated neurotoxicity and whether they would retain their neuroprotective attributes when oxycodone was present. Based on previous studies, we hypothesized that HIV-1 Tat would increase the proportion of cell death and that oxycodone could potentiate these effects. We anticipated that E_2 and P_4 would attenuate cell death in a dose-dependent manner.

2. **Materials and Methods**

The use of animals in these studies was preapproved by the Institutional Animal Care and Use Committee (IACUC) at the University of Mississippi. All experiments were conducted in accordance with ethical guidelines defined by the National Institutes of Health (NIH Publication No. 85-23).

2.1. Subjects and housing

For the *in vivo* experiments, ovariectomized (OVX), Tat transgenic, female mice (n = 13/group) expressed (Tat+), or did not express (Tat-), the HIV-1 Tat_{1-86} protein in a doxycycline-dependent manner (Paris et al., 2016; Gonek et al., 2018) and were assessed for morphine preference using two-bottle choice and precipitated withdrawal. Animals were housed in a temperature- and humidity- controlled environment on a reversed 12:12h light-dark cycle (lights on at 09:00 h) with *ad libitum* access to food and water.

2.2. Experiment 1: Morphine Two-Bottle Choice

Ovariectomized mice were singly-housed and acclimated to two drinking bottles filled with water for days 1-6. Drinking bottles were filled with 50 mL of water to habituate mice to the procedure. Mice and bottles were weighed daily and values were recorded. Bottles were replenished to 50 mL daily. On days 2-6 Tat expression was induced (or not) via daily doxycycline injection (30 mg/kg, i.p.). On days 7-11, one bottle was filled with a solution of quinine (0.1 mg/ml in water) and the other contained a solution of morphine (0.3 mg/ml in water). Bottles and mice were weighed daily, and the placement of the bottles in the cage was counterbalanced to obviate potential effects caused by a side-preference. On the last day, mice were injected with the non-selective opioid receptor antagonist,

naloxone (1 mg/kg, i.p.) to precipitate withdrawal. To assess estradiol's effect on morphine preference, self-administration, and withdrawal behaviors, ovariectomized mice were subcutaneously administered estradiol (0.09 mg/kg, s.c., QOD) throughout the study.

2.3. Experiment 2: Precipitated withdrawal

Mice were administered an injection of the opioid receptor antagonist, naloxone (1 mg/kg, s.c.), in order to precipitate withdrawal. Mice were singly-housed in a rectangular, clear observation box (16 x 16 x 30 cm) and observed for 10 minutes. The proportion of mice that jumped, latency to jumping, frequency of jumps, forepaw tremors, wet-dog shakes, and fecal boli were recorded as indices of withdrawal (Fitting et al., 2016).

2.4. Experiment 3: Neurotoxicity of HIV-1 Tat and oxycodone

2.4.1. Chemicals

SH-SY5Y human neuroblastoma cells were treated with vehicle or a saturating dose of oxycodone (500 nM in ddH_2O; Sigma-Aldrich, Saint Louis, MO), vehicle or low-to-high physiological P_4 (10 or 100 nM; Sigma-Aldrich) or E_2 (1 or 10 nM; Sigma-Aldrich; Paris et al., 2016) dissolved in DMSO and diluted 1:10,000 in media, and vehicle or HIV-1 Tat_{1-86} (100 nM diluted to concentration in ddH_2O; #1002-2, ImmunoDx, Woburn, MA) for 20 h prior to live/dead assessment. The concentration of Tat reflects one from a range that elicits functional deficits in glia and neurons similar to those observed in HIV infection (Kruman et al., 1998; Nath et al., 1999; Singh et al., 2004; El-Hage et al., 2005, 2008; Perry et al., 2010). SH-SY5Y cells were differentiated via sequential exposure to retinoic acid (1.5 mg/ml dissolved in 95% EtOH and protected from light; #R2625, Sigma-Aldrich) and

BDNF (10 µg/ml dissolved in DMEM/F12; #SRP3014, Sigma-Aldrich) as described below.

2.4.2. Cell Culture

Differentiated Human Neuroblastoma Cells (SH-SY5Y): SH-SY5Y neuroblastoma cells were obtained from ATCC (#CRL-2266, Manassas, VA). Cells were seeded onto 24-well plates at a density of 5×10^4/well for assessment of cell death. Prior to differentiation, cells were maintained in growth media: 89.5% DMEM/F12 (#11320-033, Life Technologies, Carlsbad, CA), 10% heat-inactivated fetal bovine serum (FBS; #SH30071.03, Thermo Scientific Hyclone, Logan, UT), and 0.5% antibiotic/antimycotic mixture (#15240-062, Life Technologies). One day after seeding, growth media was fully exchanged for differentiation media #1 which contained retinoic acid diluted 1:500 in growth media. After 24 hours in differentiation media #1, media was fully exchanged for the serum-free differentiation media #2 consisting of BDNF diluted 1:200 in DMEM/F12 (supplemented only with the 0.5% antibiotic/antimycotic mixture). Cells were differentiated and ready to undergo experimental manipulation the next day. On the day of the experiment, dilutions were prepared to achieve the appropriate concentrations of oxycodone (500 nM) and E_2 (1 nM 10 nM), or P_4 (10 nM,100 nM) prior to administration.

2.4.3. Live/Dead Assay

Briefly, experimental treatments were applied to differentiated SH-SY5Y cells seeded on 24-well plates and live/dead assay was performed 20 h later. Prior work utilizing time-lapsed microscopy (0-60 h) identified the 20 h time-point as the earliest time when Tat- and pregnane steroid-treated cells significantly diverged on the measure of viability

(Paris et al., 2016). To stain the cells for imaging, a working solution of propidium iodine and Hoescht was prepared by diluting stocks in Hank's Balanced Salt Solution (HBSS; 1:500 dilution). Media in the 24-well plates was replaced with HBSS containing propidium iodide and Hoescht. Cells were imaged using a Nikon Ti-2 microscope and quantified using ImageJ software. Viability of the cells was assessed by calculating the proportion of necrotic cells [# propidium iodide$^+$ cells / # total cells) *100].

2.5. Statistical analyses

In the two bottle choice experiment, liquid intake was assessed by measuring the difference in daily bottle weight and assuming a density of 1 g/ml. Total liquid intake was calculated by adding together the intake from both the test and control bottles, and preference was calculated as follows: (test bottle intake) / (total liquid intake) *100. Drug intake was calculated as: test bottle intake * test compound concentration (mg/ml) / daily weight (kg). Preference and total liquid intake were analyzed via separate repeated measures ANOVAs, and somatic signs of withdrawal were analyzed via separate two-way ANOVAs. Live/dead analyses were assessed via separate three-way ANOVAs with oxycodone condition, hormone condition, and Tat condition as factors. Fisher's Protected Least Significant Difference *post-hoc* tests determined group differences following main effects. Interactions were delineated via simple main effects and main effect contrasts with alpha controlled for multiple comparisons. Analyses were considered significant when *p* < 0.05. All statistical analyses were assessed via SAS StatView.

3. Results

3.1. Experiment 1: Estradiol increased morphine preference and total liquid intake

To assess whether Tat increased morphine preference and self-administration behaviors, and whether estradiol influences this, estradiol's effect on morphine self-administration behaviors, ovariectomized Tat induced (or not induced) mice were singly-housed and given the choice to drink from 2 different bottles: one containing a solution of quinine (0.1mg/ml in water) and the other containing a solution of morphine (0.3mg/ml in water). On day 1, vehicle-administered Tat(+) mice showed a significantly greater preference for the morphine solution than did vehicle-administered Tat(-) mice [$F(2,42) = 5.78, p < 0.05$] (Fig. 1A). However, all mice increased their preference for the morphine solution during the following days (Fig. 1A, 1B). Further, estradiol significantly increased total liquid intake in both Tat(+) and Tat(-) mice [$F(2,42) = 13.787, p < 0.05$] (Fig. 1C), compared to vehicle mice (fig. 1D). Lastly, estradiol also significantly increased consumption of morphine in all mice, irrespective of Tat exposure [$F(2,42) = 13.787, p < 0.05$](Fig. 1E) compared to vehicle administration (Fig. 1F).

3.2. Experiment 2: Tat or estradiol increased withdrawal behavior

The purpose of this experiment was to examine estradiol's effect on morphine-mediated withdrawal behaviors in ovariectomized mice. To precipitate withdrawal, mice were administered an injection of naloxone, and the number jumps, latency to jump, forepaw tremors, wet-dog shakes, and fecal boli were recorded and analyzed to quantify withdrawal behaviors. Tat expression significantly increased jumping behavior compared to Tat(-) controls [$F(4,26) = 1.94, p < 0.05$] (Fig. 2A), which is the primary indication of

withdrawal within rodent models. Estradiol also significantly increased jumping behavior in Tat (-) control mice [$F(4,26 = 1.940, p = 0.017$] (Fig. 2A). Notably, estradiol appeared to attenuate jumping among Tat (+) mice, but this was not statistically significant ($p = 0.07$) (Fig. 2A). Additionally, estradiol appeared to attenuate the latency to jump, but this did not reach significance (Fig. 2B).

3.3. Experiment 3: Neurotoxicity of HIV-1 Tat and oxycodone

3.3.1. Neuroprotective Effects of Estradiol on Tat-mediated Cytotoxicity

To assess the neuroprotective potential of estradiol on HIV-1 Tat-mediated cell death, differentiated SH-SY5Y cells were treated with vehicle, oxycodone (500 nM), Tat (100 nM), and/or E_2 (1 nM or 10 nM). A three-way ANOVA revealed a significant hormone condition x Tat condition interaction [$F(2,155) = 3.36, p < 0.05$] (Fig. 3A). Exposure to Tat significantly increased the proportion of cell death compared to vehicle-treated control wells ($p = 0.0001$). Irrespective of oxycodone administration, E_2 administered at 1 nM ($p = 0.047$) or 10 nM ($p = 0.026$) significantly attenuated Tat-mediated cell death. No additional effect of oxycodone was observed on cell viability.

3.3.2. Neuroprotective Effects of Progesterone on Tat-mediated Cytotoxicity

To assess the neuroprotective potential of progesterone on HIV-1 Tat-mediated cell death, differentiated SH-SY5Y cells were treated with vehicle, oxycodone (500 nM), Tat (100 nM), and/or P_4 (10 nM or 100 nM). A three-way ANOVA revealed a significant hormone condition $\times$ Tat condition interaction [$F(2, 192) = 3.72, p < 0.05$] (Fig. 3B). Exposure to Tat significantly increased the proportion of cell death compared to vehicle-treated control wells ($p = 0.003$). Irrespective of oxycodone administration, P_4 administered

at 100 nM ($p = 0.003$) significantly attenuated Tat-mediated cell death. P_4 administered at 10 nM failed to produce a significant attenuation of Tat-mediated cell death ($p = 0.18$, *n.s.*).

No additive effect of oxycodone was observed on cell viability.

4. **Discussion**

The findings of Experiment 1 in the present study upheld the hypothesis that estradiol would potentiate HIV-1 Tat-mediated morphine reinforcement in ovariectomized mice. On the first day of morphine administration, Tat significantly increased the acquisition of a morphine preference among vehicle-treated mice compared to Tat(-) vehicle-administered controls. Interestingly, estradiol significantly increased, not only morphine consumption, but total liquid intake as well, irrespective of Tat exposure. The findings of Experiment 2 upheld the hypothesis that estradiol would increase withdrawal behaviors. Tat expression significantly increased jumping behaviors in vehicle-administered mice. Similarly, E_2 significantly increased jumping behaviors in Tat(-) mice. Albeit, we noted the non-significant appearance of attenuated jumping behavior in Tat(+) mice administered E_2. Estradiol also non-significantly decreased the latency to jump for all mice. Lastly, the aims of Experiment 3 were to determine whether oxycodone influenced HIV-1 Tat-mediated neurotoxicity in differentiated SH-SY5Y cells, to investigate whether administration of E_2 and P_4 would attenuate these neurotoxic effects, and whether these neuroprotective attributes would be observed in a dose-dependent manner. The findings from the present experiment upheld the hypothesis that exposure to HIV-1 Tat would increase the proportion of cell death in differentiated SH-SY5Y human neuroblastoma cells. Oxycodone did not potentiate HIV-1 Tat-mediated neurotoxicity. Additionally, administration of E_2 significantly attenuated Tat-mediated neurotoxicity at both concentrations (1 nM, 10 nM). Administration of P_4 only significantly attenuated Tat-mediated neurotoxicity at the larger concentration (100 nM). These findings confirm and extend previous work.

14

The current literature suggests that sex differences in the addiction cycle exist, yet females remain underrepresented in addiction research. For example, preclinical studies show that females tend to acquire self-administration faster and self-administer higher amounts of drugs than do males (Lynch & Carroll, 1999). One factor that is thought to underlie these gender disparities are sex hormones, specifically estradiol. In fact, studies show that female rats will work harder for and self-administer higher doses of cocaine more during the estrous phase, when estradiol is highest, than any other phase in the estrous cycle (Roberts et al., 1989). Further, when ovariectomized rats are treated with estradiol, the motivation to self-administer cocaine is enhanced (Lynch et al., 2001).Studies show that estradiol may work in concert with the dopamine reward circuitry to potentiate the rewarding properties of drugs, thus increasing their abuse potential in females (Becker, 1999; Yoest et al., 2014). Consistent with the current literature, we observed a significant increase in morphine consumption among E_2-treated subjects in the present study Though it is important to note that we used ovariectomized mice in these experiments. The use of ovariectomized animals allows the manipulation of hormones, but may also present a potential caveat in that it could affect sensitivity to E_2. Nonetheless, further investigation is needed to determine estradiol's behavioral effects in concert with opioids on the HIV-positive female population, as the effects of opioids are not well understood.

Substance abuse and HIV-1 infection are well recognized as comorbid epidemics. Comorbid substance abuse has been known to reduce the time for HIV-1 patients to develop neuroHIV symptoms, or even worsen the neurological complications of neuroHIV (Bell et al., 2006). Past studies suggest that the infiltration of the dopaminergic system by HIV-1 proteins such as Tat, and subsequent Tat-mediated dysfunction may underlie some

of these symptoms (Fitting et al., 2015). As the dopaminergic system is responsible for drug reward and reinforcement, this biologically implies that the presence of Tat may possess some influence over the rewarding properties of certain drugs. Furthermore, this effect may be observed in psychostimulant drugs, as one study revealed that HIV-1 Tat-induced mice display significant increases in cocaine-conditioned place preference, as well as longer cocaine-induced hyperlocomotion than non-induced male mice (Paris et al., 2014). Though this is apparent in psychostimulants, Tat's behavioral effects in conjunction with opioid drugs remain little known. Although, evidence from another study suggests this potentiated effect may also be observed with morphine administration (Gonek et al., 2018). In the present study, we observed a significant increase in morphine preference on the first day of the two-bottle choice protocol in Tat-induced, ovariectomized female mice. This finding suggests that Tat decreased the time to acquire morphine self-administration, thus potentially alluding to Tat-mediated potentiation of the rewarding properties of morphine.

A typical cycle of addiction commonly includes multiple periods of abstinence, withdrawal, and subsequent relapse. Sex differences are still apparent in these late stages of addiction as well. Some drugs cause women to experience worsened withdrawal symptoms than men (Becker & Koob, 2016). Generally, females also experience greater sensitivity to stress during the abstinence stage, which may explain the higher tendency of women to relapse compared to men (Hudson & Stamp, 2011). These effects remain consistent when modeled in animals (Becker & Koob, 2016). However, HIV-1 Tat and estradiol's concurrent effect on morphine dependence are poorly understood. In the current study, we aimed to measure this by utilizing a precipitated withdrawal procedure. In this

experiment, we observed a significant Tat-mediated increase in the jumping behaviors of morphine dependent mice. Estradiol further increased these jumping behaviors in control mice; while displaying a tendency to decrease these jumping behaviors in Tat-induced mice. These findings suggest that Tat and estradiol may potentiate morphine dependence when administered and expressed separately; however, potentially protective interactions between estradiol and Tat cannot be ruled out. This finding could likely be in agreement with *in vitro* studies of estradiol neuroprotection, but further examination is needed.

HIV-1 associated neurocognitive deficits are partly mediated by neuroinflammation in the CNS due to an upregulation of cytokines caused by viral HIV-1 proteins. This neuroinflammation contributes to the neurotoxicity of HIV-1 Tat. However, there is evidence that pregnane and estrane hormones may be able to protect against this neurotoxicity. For example, an *in vitro* study revealed that allopregnanolone (a metabolite of P_4) can protect against the neurotoxic effects of HIV-1 Tat in neuronal-glial co-cultures, while also attenuating the increase of microglial activation caused by exposure to Tat (Paris et al., 2016, 2020). The present study was conducted on neuroblastoma cells in the absence of glial inputs in order to parse the effects on neuron without Tat-potentiation of glially-mediated cytokine production. E_2 significantly attenuated Tat-mediated cell death at both concentrations (1 nM, 10 nM), while P_4 significantly attenuated cell death at the larger concentration (100 nM). Though the 10 nM concentration of P_4 did not produce a significant effect, there appeared to be a trend that led to less cell death in this condition. These findings were consistent with evidence from past studies that suggest that estrane and pregnane have neuroprotective qualities (Kendall, 2005; Wallace et al., 2006). These data from the current study and past studies suggest that these steroid hormones may

ameliorate the prevalence of neurocognitive deficits by attenuating the neurotoxic effects of HIV proteins such as Tat. More studies should be conducted to further investigate their neuroprotective qualities.

The intertwined epidemics of intravenous drug use and new HIV-1 cases caused by injection drug use significantly increases the urgency to understand the interaction between opioids and HIV-1. Findings in the current literature of opioid's effects on HIV-1 are discrepant. Some studies suggest that opioids, such as morphine, may have protective qualities against HIV-1 *in vivo* (Donahoe et al., 2009). Other studies suggest that morphine stimulates replication of viral HIV proteins (Schweitzer et al., 1991), and also interacts with HIV-1 Tat to potentiate its neurotoxic effects *in vitro* (Fitting et al., 2014). Effects of opioids can be biphasic, exerting different actions acutely vs. those observed chronically. In support, opioids are initially immunosuppressive, but promote a profound immune response in withdrawal (Gonek et al., 2018). Though opioid-HIV interactions have now been the subject of research for nearly 20 years, interactions between oxycodone, a commonly prescribed opioid analgesic, and HIV-1 remain under-investigated. This interaction is important to understand due to the higher prevalence of long-term opioid usage among HIV patients (Silverberg et al., 2012). In the current study, oxycodone in combination with HIV-1 Tat did not elicit any significant effects or interactions in differentiated SH-SY5Y cells. However, one limitation is that this interaction was solely examined in neuronal cells. Previous literature suggests that glia cells are critical to opiate responses (El-Hage et al., 2008; Hauser et al., 2012). This suggests that further investigation is warranted to determine whether these results will remain consistent in glial cells.

In sum, these data further demonstrate HIV-1 Tat's ability to modulate the behavioral effects of morphine addiction. Tat was shown to potentiate morphine self-administration acquisition and dependence; however, its presence did not interact *in vitro* with oxycodone when glia was absent. Additionally, estradiol increased morphine self-administration behavior despite Tat condition, and increased jumping behaviors in Tat (-) control mice. Interestingly, E_2 displayed protective qualities by decreasing jumping behaviors when administered to Tat-induced, ovariectomized female mice. Further, estradiol, as well as progesterone, also displayed neuroprotective properties in culture by ameliorating Tat-mediated neurotoxicity. These findings suggest that E_2 may possess protective qualities at the cellular level, but caution must be taken when considering its capacity to increase opioid reinforcement at the behavioral level.

References

Anthony, I.C., Arango, J.C., Stephens, B., Simmonds, P., Bell, J.E. (2008). The effects of
    illicit drugs on the HIV infected brain. *Front Biosci*,13, 1294–1307.

Becker, J. B. (2016). Sex differences in addiction. *Dialogues in clinical
    neuroscience*, *18*(4), 395–402.

Becker, J.B. (1990). Direct effect of 17 β-estradiol on striatum: sex differences in
    dopamine release. *Synapse,* 5(2), 157–164.

Becker, J.B. (1999). Gender differences in dopaminergic function in striatum and nucleus
    accumbens. *Pharmacology Biochemistry and Behavior*, 64, 803–812.

Becker, J. B., & Koob, G. F. (2016). Sex Differences in Animal Models: Focus on
    Addiction. *Pharmacological reviews*, *68*(2), 242–263.
    https://doi.org/10.1124/pr.115.011163

Becker, J. B., McClellan, M. L., & Reed, B. G. (2017). Sex differences, gender and
    addiction. *Journal of neuroscience research*, *95*(1-2), 136–147.
    https://doi.org/10.1002/jnr.23963

Bertrand, S.J., Aksenova, M.V., Mactutus, C.F., Booze, R.M. (2013). HIV-1 Tat protein
    variants: critical role or the cysteine region in synaptodendritic injury. *Exp
    Neurol*, 248, 228–235.

Bell, J.E., Arango, J. & Anthony, I.C. (2006). Neurobiology of multiple insults: HIV-1
    associated brain disorders in those who use illicit drugs. *Jrnl NeuroImmune
    Pharm*, 1, **1**82–191. https://doi.org/10.1007/s11481-006-9018-2

Bucci, M. (2015). Viral mechanisms: Tat modulates DAT. *Nat Chem Biol*, 11, 240.

Cabrera-Muñoz, E., Hernández-Hernández, O. T., Camacho-Arroyo, I. (2012a). Role of estradiol and progesterone in HIV susceptibility and disease progression. *Mini-Rev Med Chem,* 12, 1049–1054.

Cummings, J.A., Jagannathan, L., Jackson, L.R., Becker, J.B. (2014). Sex differences in the effects of estradiol in the nucleus accumbens and striatum on the response to cocaine: neurochemistry and behavior. *Drug Alcohol Depend,* 135, 22–28.

Donahoe, R.M., O'Neil, S.P., Marsteller, F.A., Novembre, F.J., Anderson, D.C., Lankford-Turner, P., McClure, H.H. (2009). Probable deceleration of progression of simian AIDS affected by opiate dependency: studies with a rhesus macaque/SIVsmm9 model. *J Acquir Immune Defic. Syndr*, 50, 241–249.

Dutta, R., & Roy, S. (2012). Mechanism(s) involved in opioid drug abuse modulation of HAND. *Current HIV research*, *10*(5), 469–477. https://doi.org/10.2174/157016212802138805

Edelman, E. J., Gordon, K., Becker, W. C., Goulet, J. L., Skanderson, M., Gaither, J. R., Brennan Braden, J., Gordon, A. J., Kerns, R. D., Justice, A. C., & Fiellin, D. A. (2013). Receipt of Opioid Analgesics by HIV-Infected and Uninfected Patients. *Journal of General Internal Medicine*, *28*(1), 82–90. https://doi.org/10.1007/s11606-012-2189-z

El-Hage, N., Gurwell, J.A., Singh, I.N., Knapp, P.E., Nath, A., Hauser, K.F. (2005). Synergistic increases in intracellular Ca2+, and the release of MCP-1, RANTES, and IL-6 by astrocytes treated with opiates and HIV-1 Tat. *Glia*, 50, 91-106.

El-Hage, N., Bruce-Keller, A.J., Yakovleva, T., Bazov, I., Bakalkin, G., Knapp, P.E., Hauser, K.F. (2008). Morphine exacerbates HIV-1 Tat-induced cytokine production in astrocytes through convergent effects on [Ca(2+)](i), NF-kappaB trafficking and transcription. *PLoS One,* 3, e4093.

Fitting, S., Booze, R. M., & Mactutus, C. F. (2015). HIV-1 proteins, Tat and gp120, target the developing dopamine system. *Current HIV research*, *13*(1), 21–42. https://doi.org/10.2174/1570162x13666150121110731

Fitting, S., Xu, R., Bull, C., Buch, S. K., El-Hage, N., Nath, A., Knapp, P. E., & Hauser, K. F. (2010). Interactive comorbidity between opioid drug abuse and HIV-1 Tat: chronic exposure augments spine loss and sublethal dendritic pathology in striatal neurons. *The American journal of pathology*, *177*(3), 1397–1410. https://doi.org/10.2353/ajpath.2010.090945

Fitting, S., Zou, S., El-Hage, N., Suzuki, M., Paris, J.J., Schier, C.J., Rodriguez, J.W., Rodriguez, M., Knapp, P.E., Hauser, K.F. (2014). Opiate addiction therapies and HIV-1 Tat: interactive effects on glial [Ca(2)(+)]i, oxyradical and neuroinflammatory chemokine production and correlative neurotoxicity. *Curr HIV Res*, 12(6), 424–434.

Fitting, S., Knapp, P.E., Zou, S., Marks, W.D., Bowers, M.S., Akbarali, H.I., Hauser, K.F. (2014). Interactive HIV-1 Tat and morphine-induced synaptodendritic injury is triggered through focal disruptions in Na$^+$ influx, mitochondrial instability, and Ca^{2+} overload. *J Neurosci*, 17, 34(38), 12850-64.

Fitting, S., Stevens, D.L., Khan, F.A., Scoggins, K.L., Enga, R.M., Beardsley, P.M., Knapp, P.E., Dewey, W.L., Hauser, K.F. (2016). Morphine tolerance and physical dependence are altered in conditional HIV-1 Tat transgenic mice. *J. Pharmacol. Exp. Ther,* 356, 96-105.

Fitting, S., Ignatowska-Jankowska, B.M., Bull, C., Skoff, R.P., Lichtman, A.H., Wise, L.E., Fox, M.A., Su, J., Medina, A.E., Krahe, T.E., Knapp, P.E., Guido, W., Hauser, K.F. (2013). Synaptic dysfunction in the hippocampus accompanies learning and memory deficits in human immunodeficiency virus type-1 Tat transgenic mice. *Biol Psychiatry,* 73, 443-53.

Gaskill, P. J., Calderon, T. M., Coley, J. S., & Berman, J. W. (2013). Drug induced increases in CNS dopamine alter monocyte, macrophage and T cell functions: implications for HAND. *Journal of neuroimmune pharmacology : the official journal of the Society on NeuroImmune Pharmacology*, *8*(3), 621–642. https://doi.org/10.1007/s11481-013-9443-y

Gonek, M., McLane, V.D., Stevens, D.L., Lippold, K., Akbarali, H.I., Knapp, P.E., Dewey, W.L., Hauser, K.F., Paris, J.J. (2018). CCR5 mediates HIV-1 Tat-induced neuroinflammation and influences morphine tolerance, dependence, and reward. *Brain Behav Immun*, 69, 124-138.

Gorman, M. (1998). A tale of two epidemics: HIV and stimulant use. *Focus,* 13, 1–3.

Gras, G., Chretien, F., Vallat-Decouvelaere, A.V., et al. (2006). Regulated expression of sodium dependent glutamate transporters and synthetase: a neuroprotective role

for activated microglia and macrophages in HIV infection? *Brain Pathology*, *13*(2), 211–222.

Haughey, N.J., Holden, C.P., Nath, A. (1999). Involvement of inositol 1,4,5 trisphosphate regulated stores of intracellular calcium in calcium dysregulation and neuron cell death caused by HIV-1 protein tat. *Journal of Neurochemistry*, *73*(4), 1363-1374.

Haughey, N.J. and Mattson, M.P. (2002). Calcium dysregulation and neuronal apoptosis by the HIV-1 proteins Tat and gp120. *J Acquir Immune Defic Syndr*, 31, 55-61.

Hauser, K.F., Fitting, S., Dever, S.M., Podhaizer, E.M., Knapp, P.E. (2012). Opiate drug use and the pathophysiology of neuroAIDS. *Curr HIV Res,* 10, 435–452.

Hauser, K.F., Hahn, Y.K., Adjan, V.V., Zou, S., Buch, S.K., Nath, A., Bruce-Keller, A.J., Knapp, P.E. (2009). HIV-1 Tat and morphine have interactive effects on oligodendrocyte survival and morphology. *Glia,* 57, 194-206.

Hahn, Y.K., Podhaizer, E.M., Farris, S.P., Miles, M.F., Hauser, K.F., Knapp, P.E. (2015). Effects of chronic HIV-1 Tat exposure in the CNS: heightened vulnerability of males versus females to changes in cell numbers, synaptic integrity, and behavior. *Brain Struct. Funct*, 220, 605-623

Hudson, A., Stamp, J.A. (2011). Ovarian hormones and propensity to drug relapse: a review. *Neuroscience and Biobehavioral Reviews*, 35(3), 427–436.

Hu, M., Becker, J.B. (2003). Effects of sex and estrogen on behavioral sensitization to cocaine in rats. *J Neurosci,* 23(2), 693–699.

Hu, X. T. (2016). HIV-1 Tat-mediated calcium dysregulation and neuronal dysfunction in vulnerable brain regions. *Current drug targets*, 17(1), 4–14.

Kalivas, P.W., Volkow, N.D. (2005). The neural basis of addiction: a pathology of motivation and choice. *Am J Psychiatry*, 162, 1403–1413.

Kendall, S.L., Anderson, C.F., Nath, A., Turchan-Cholewo,J., Land, C.L., Mactutus, C.F., Booze, R.M. (2008). Gonadal steroids differentially modulate neurotoxicity of HIV and cocaine: testosterone and ICI 182,780 sensitive mechanism. *BMC Neurosci,* 6, 40.

Kesby, J.P., Fields, J.A., Chang, A., Coban, H., Achim, C.L., Semenova, S., TMARC Group. (2018). Effects of HIV-1 TAT protein and methamphetamine exposure on visual discrimination and executive function in mice. *Behav Brain Res*, *3*(349), 73-79.

Kesby, J. P., Markou, A., & Semenova, S. (2016). The effects of HIV-1 regulatory TAT protein expression on brain reward function, response to psychostimulants and delay-dependent memory in mice. *Neuropharmacology*, *109*, 205–215. https://doi.org/10.1016/j.neuropharm.2016.06.011

Koob, G. F., & Volkow, N. D. (2016). Neurobiology of addiction: a neurocircuitry analysis. *The lancet. Psychiatry*, *3*(8), 760–773. https://doi.org/10.1016/S2215-0366(16)00104-8.

Kruman, I.I., Nath, A., Mattson, M.P. (1998). HIV-1 protein Tat induces apoptosis of hippocampal neurons by a mechanism involving caspase activation, calcium overload, and oxidative stress. *Exp Neurol, 154*, 276-88.

Larson, E. B., Anker, J. J., Gliddon, L. A., Fons, K. S., & Carroll, M. E. (2007). Effects

    of estrogen and progesterone on the escalation of cocaine self-administration in

    female rats during extended access. *Experimental and Clinical*

    Psychopharmacology, 15(5), 461–471. https://doi.org/10.1037/1064-

    1297.15.5.461

Li, T.S., Tubiana, R., Katlama, C., Calvez, V., Ait Mohand, H., Autran, B. (1998). Long-

    lasting recovery in CD4 T-cell function and viral-load reduction after highly

    active antiretroviral therapy in advanced HIV-1 disease. *Lancet*, *351,* 1682–6.

Lynch, W.J., Carroll, M.E. (1999). Sex differences in the acquisition of intravenously

    self-administered cocaine and heroine in rats. *Psychopharmacology (Berl)*, *144*,

    77–82

Lynch, W.J., Roth, M.E., Mickelberg, J.L., Carroll, M.E. (2001). Role of estrogen in the

    acquisition of intravenously self-administered cocaine in female

    rats. *Pharmacology Biochemistry and Behavior*, *68*, 641–646.

Lopes, M., Olfson, M., Rabkin, J., Hasin, D.S., Alegría, A.A., Lin, K.H., Grant, B.F.,

    Blanco, C. (2012) Gender, HIV status, and psychiatric disorders: results from the

    National Epidemiologic Survey on Alcohol and Related Conditions. *J. Clin.*

    Psychiatry, 73, 384–391.

Marcus, K.S., Kerns, R.D., Rosenfeld, B., Breitbart, W. (2000). HIV/AIDS-related pain

    as a chronic pain condition: Implications of a biopsychosocial model for

    comprehensive assessment and effective management. *Pain Med, 1*, 260–273.

McLean, C. P., Asnaani, A., Litz, B. T., & Hofmann, S. G. (2011). Gender differences in
anxiety disorders: prevalence, course of illness, comorbidity and burden of
illness. *Journal of psychiatric research*, *45*(8), 1027–1035.
https://doi.org/10.1016/j.jpsychires.2011.03.006

Merlin, J.S., Tamhane, A., Starrels, J.L., Kertesz, S., Saag, M., Cropsey, K. (2016).
Factors associated with prescription of opioids and co-prescription of sedating
medications in individuals with HIV. *AIDS Behav, 20*(3), 687-98.

Miaskowski, C., Penko, J.M., Guzman, D., Mattson, J.E., Bangsberg, D.R., Kushel, M.B.
(2011). Occurrence and characteristics of chronic pain in a community-based
cohort of indigent adults living with HIV infection. *J Pain*, *12*, 1004-1016.

Midde, N. M., Gomez, A. M., & Zhu, J. (2012). HIV-1 Tat protein decreases dopamine
transporter cell surface expression and vesicular monoamine transporter-2
function in rat striatal synaptosomes. *Journal of neuroimmune pharmacology: the
official journal of the Society on NeuroImmune Pharmacology*, *7*(3), 629–639.
https://doi.org/10.1007/s11481-012-9369-9

Nakagawa, F., May, M., Phillips, A. (2013). Life expectancy living with HIV: Recent
estimates and future implications. *Curr Opin Infect Dis*, *26,* 17–25.

Nath, A., Conant, K., Chen, P., Scott, C., Major, E.O. (1999). Transient exposure to HIV-
1 Tat protein results in cytokine production in macrophages and astrocytes. A hit
and run phenomenon. *J Biol Chem, 274*, 17098-102.

Paris, J. J., Fenwick, J., & McLaughlin, J. P. (2014). Progesterone protects normative
anxiety-like responding among ovariectomized female mice that conditionally

express the HIV-1 regulatory protein, Tat, in the CNS. *Hormones and behavior*, *65*(5), 445–453. https://doi.org/10.1016/j.yhbeh.2014.04.001

Paris, J.J., Carey, A.N., Shay, C.F., Gomes, S.M., He, J.J., McLaughlin, J.P. (2014). Effects of conditional central expression of HIV-1 tat protein to potentiate cocaine-mediated psychostimulation and reward among male mice. *Neuropsychopharmacology*, *39,* 380-8.

Paris, J.J., Zou, S., Hahn, Y.K., Knapp, P.E., Hauser, K.F. (2016). 5α-reduced progestogens ameliorate mood-related behavioral pathology, neurotoxicity, and microgliosis associated with exposure to HIV-1 Tat. *Brain Behav Immun*, *55,* 202-214.

Paris, J. J., Liere, P., Kim, S., Mahdi, F., Buchanan, M. E., Nass, S. R., Qrareya, A. N., Salahuddin, M. F., Pianos, A., Fernandez, N., Shariat-Madar, Z., Knapp, P. E., Schumacher, M., & Hauser, K. F. (2020). Pregnane steroidogenesis is altered by HIV-1 Tat and morphine: Physiological allopregnanolone is protective against neurotoxic and psychomotor effects. *Neurobiology of stress*, *12*, 100211. https://doi.org/10.1016/j.ynstr.2020.100211

Peris, J., Decambre, N., Coleman-Hardee, M.L., Simpkins, J.W. (1991). Estradiol enhances behavioral sensitization to cocaine and amphetamine-stimulated striatal [^{3}H]dopamine release. *Brain Res, 566*(1-2):255–264

Perl, A., Banki, K. (2000). Genetic and metabolic control of the mitochondrial transmembrane potential and reactive oxygen intermediate production in HIV disease. *Antioxidants & Redox Signaling, 2*(3):551–573

Perry, S.W., Barbieri, J., Tong, N., Polesskaya, O., Pudasaini, S., Stout, A., Lu, R., Kiebala, M., Maggirwar, S.B., Gelbard, H.A. (2010). Human immunodeficiency virus-1 Tat activates calpain proteases via the ryanodine receptor to enhance surface dopamine transporter levels and increase transporter-specific uptake and Vmax. *J Neurosci, 30,* 14153-64.

Roberts, D.C., Bennett, S.A., Vickers, G.J. (1989). The estrous cycle affects cocaine self-administration on a progressive ratio schedule in rats. *Psychopharmacology (Berl), 98,* 408–11.

Sacktor, N., Haughey, N., Cutler, R., Tamara, A., Turchan, J., Pardo, C., Vargas, D., Nath, A. (2004). Novel markers of oxidative stress in actively progressive HIV dementia. *Journal of Neuroimmunology*, 157(1-2), 176–184.

Salahuddin, M. F., Qrareya, A. N., Mahdi, F., Jackson, D., Foster, M., Vujanovic, T., … Paris, J. J. (2020). Combined HIV-1 Tat and oxycodone activate the hypothalamic-pituitary-adrenal and -gonadal axes and promote psychomotor, affective, and cognitive dysfunction in female mice. *Hormones and Behavior*, *119*. https://doi.org/10.1016/j.yhbeh.2019.104649

Schweitzer, C., Keller, F., Schmitt, M.P., Jaeck, D., Adloff, M., Schmitt, C., Royer, C., Kirn, A., Aubertin, A.M. (1991). Morphine stimulates HIV replication in primary cultures of human Kupffer cells. *Res Virol*, *142,* 189–195.

Sewell, M.C., Goggin, K.J., Rabkin, J.G., Ferrando, S.J., Mcelhiney, M.C., Evans, S. (2000). Anxiety syndromes and symptoms among men with AIDS: A Longitudinal controlled study. *Psychosomatics*, *41*(4), 294–300.

Simioni, S., Cavassini, M., Annoni , J.M., Rimbault Abraham, A., Bourquin, I., Schiffer,

    V., Calmy, A., Chave, J.P., Giacobini, E., Hirschel, B., Du Pasquier, R.A. (2010).

    Cognitive dysfunction in HIV patients despite longstanding suppression of

    viremia. *AIDS*, *24*, 1243–50.

Silverberg, M.J., Ray, G.T., Saunders, K., Rutter, C.M., Campbell, C.I., Merrill, J.O.,

    Sullivan, M.D., Banta-Green, C.J. Von, Korff, M., Weisner, C. (2012)

    Prescription long-term opioid use in HIV-infected patients. *Clin J Pain*, *1*, 39-46.

Silvers, J. M., Aksenova, M. V., Aksenov, M. Y., Mactutus, C. F., & Booze, R. M.

    (2007). Neurotoxicity of HIV-1 Tat protein: involvement of D1 dopamine

    receptor. *Neurotoxicology*, *28*(6), 1184–1190.

    https://doi.org/10.1016/j.neuro.2007.07.005

Singh, I.N., Goody, R.J., Dean, C., Ahmad, N.M., Lutz, S.E., Knapp, P.E., Nath, A.,

    Hauser, K.F. (2004). Apoptotic death of striatal neurons induced by human

    immunodeficiency virus-1 Tat and gp120: Differential involvement of caspase-3

    and endonuclease G. *J Neurovirol*, *10*, 141-51.

Tozzi, V., Balestra, P., Bellagamba, R., Valerio, Corpolongo, A., Salvatori, M., Visco-

    Comandini, U., Vlassi, C., Giulianelli, M., Galgani, S., Antinori, A., Narciso, P.

    (2007). Persistence of neuropsychologic deficits despite long-term highly active

    antiretroviral therapy in patients with HIV-related neurocognitive impairment:

    prevalence and risk factors. *J Acquir Immune Defic Syndr, 45*, 174–182.

Tsao, J.C.I., Dobalian, A., Moreau, C., Dobalian, K. (2004). Stability of anxiety and depression in a national sample of adults with human immunodeficiency virus. *The Journal of Nervous and Mental Disease*, *192*(2), 111–118.

Turchan, J., Anderson, C., Hauser, K.F., Sun, Q., Zhang, J., Liu, Y., Wise, P.M., Kruman, I., Maragos, W., Mattson, M.P., Booze, R., Nath, A. (2001). Estrogen protects against the synergistic toxicity by HIV proteins, methamphetamine and cocaine. *BMC Neurosci*, *2*(3), https://doi.org/10.1186/1471-2202-2-3

Wallace, D.R., Dodson, S., Nath, A., Booze, R.M. (2006). Estrogen attenuates gp120- and tat1-72-induced oxidative stress and prevents loss of dopamine transporter function. *Synapse*, *59*, 51–60.

Williams, A. R., & Bisaga, A. (2016). From AIDS to Opioids - How to combat an epidemic. *The New England journal of medicine*, *375*(9), 813–815. https://doi.org/10.1056/NEJMp1604223

Yoest, K. E., Cummings, J. A., & Becker, J. B. (2014). Estradiol, dopamine and motivation. *Central nervous system agents in medicinal chemistry*, *14*(2), 83–89. https://doi.org/10.2174/1871524914666141226103135

Zou, S., Fuss, B., Fitting, S., Hahn, Y.K., Hauser, K.F., Knapp, P.E. (2015). Oligodendrocytes are targets of HIV-1 Tat: NMDA and AMPA receptor-mediated effects on survival and development. *J Neurosci*, *35*, 11384-98.
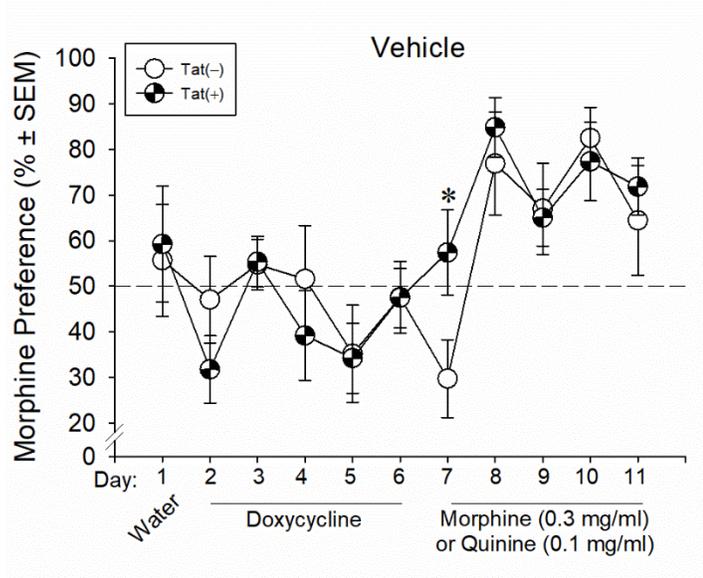
Figure 1A. Morphine preference in vehicle groups. On the first day of morphine administration, Tat significantly increased morphine preference in non-estradiol (E_2) administered mice ($p < 0.05$; n = 13/group).
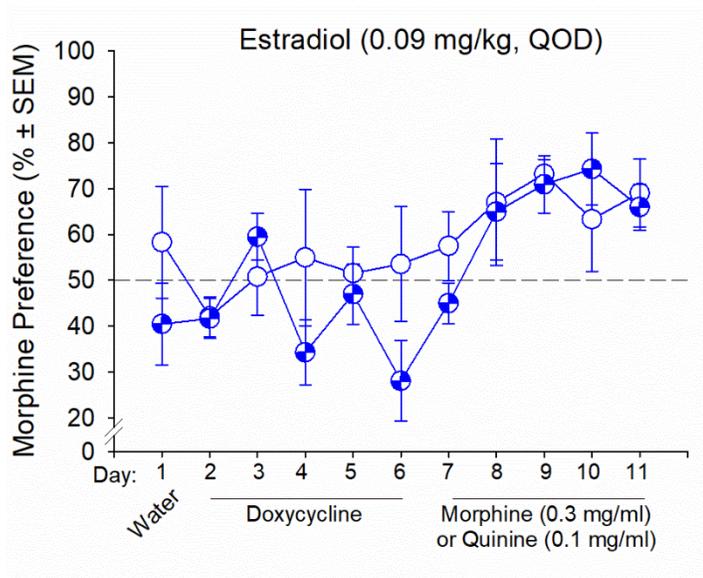

Figure 1B. Morphine preference in the estradiol group. There were no significant effects of estradiol on morphine preference (n = 13/group).

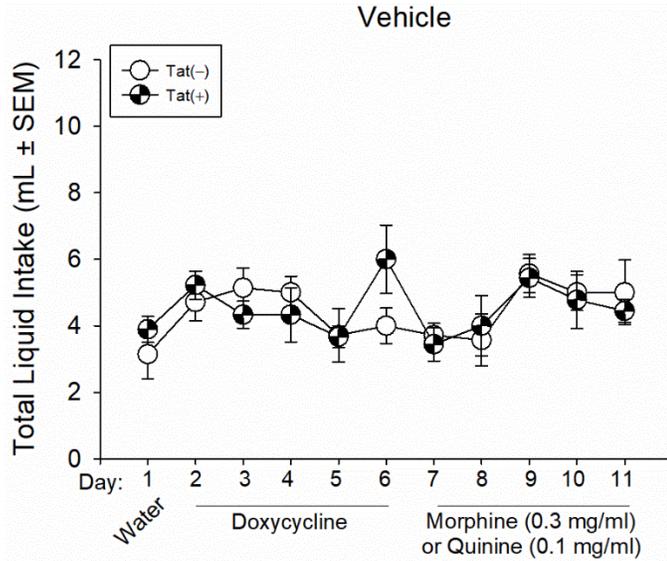**Figure 1C.** Total liquid intake in vehicle groups. There were no significant effects on liquid intake on mice that received vehicle treatments (n = 13/group).
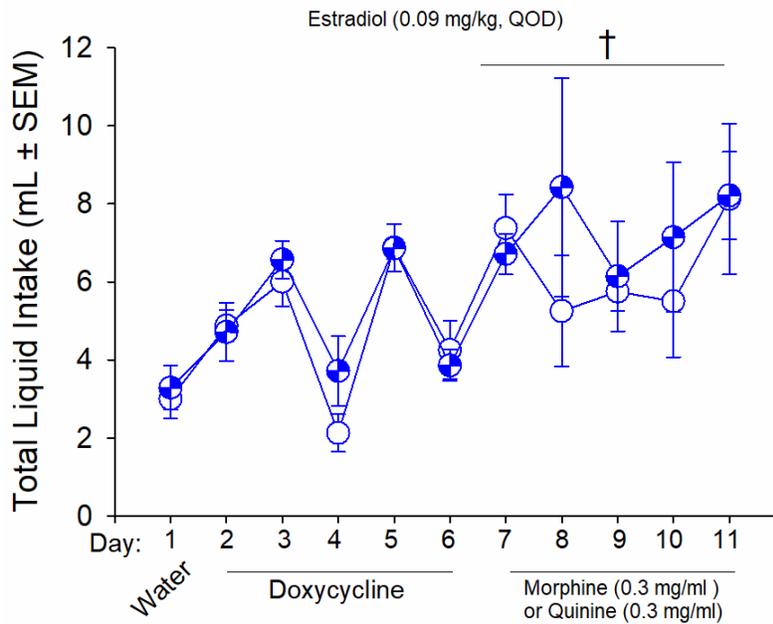
Figure 1D. Total liquid intake in estradiol groups. Estradiol significantly increased liquid intake irrespective of Tat condition ($p < 0.05$; n = 13/group).
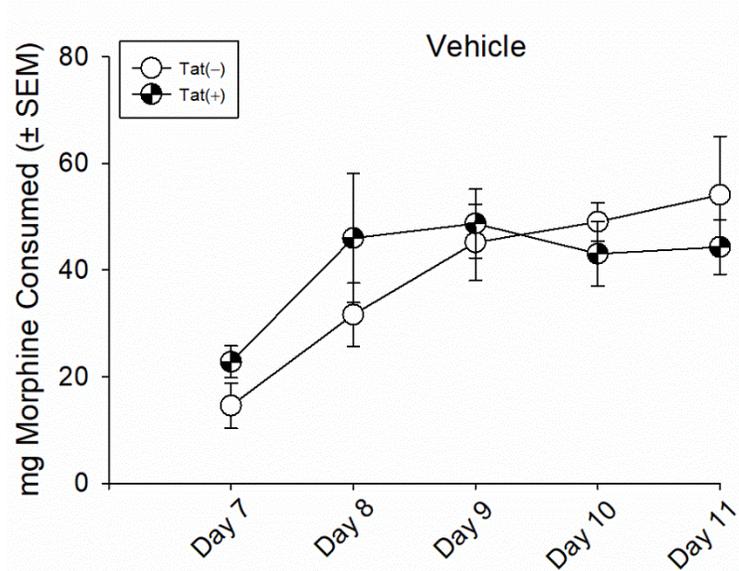
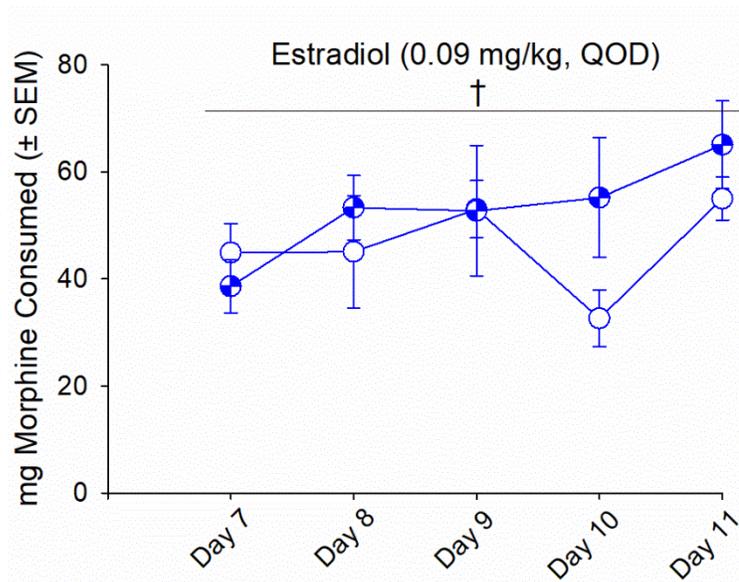**Figure 1E.** Amount of morphine consumed in vehicle groups. There were no significant effects on morphine consumption in vehicle mice (n = 13/group).

Figure 1F. Amount of morphine consumed in estradiol groups. Estradiol administration significantly increased morphine consumption, irrespective of Tat condition ($p < 0.05$; n = 13/group).
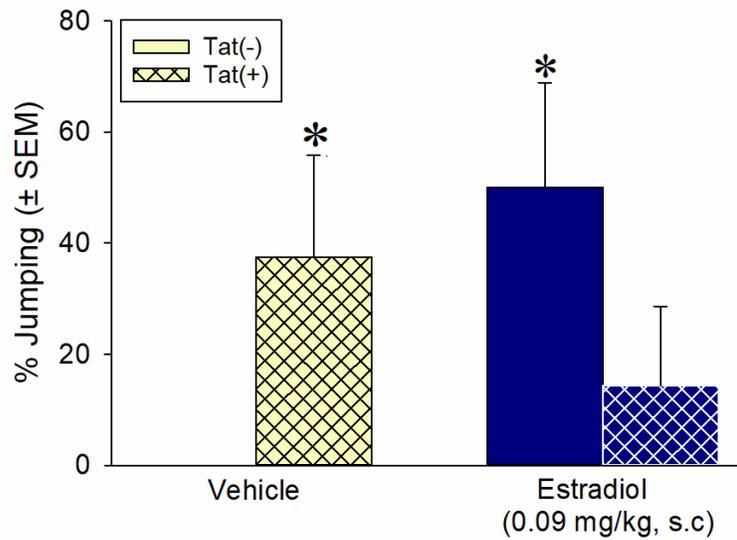
Figure 2A. Percent jumping in vehicle and estradiol groups. Tat significantly increased jumping behaviors in non-estradiol (E2) administered mice ($p < 0.05$). Estradiol significantly increased jumping behavior in Tat (-) mice ($p = 0.017$), and non-significantly attenuated jumping behavior in Tat (+) mice ($p = 0.0669$; n = 13/group).
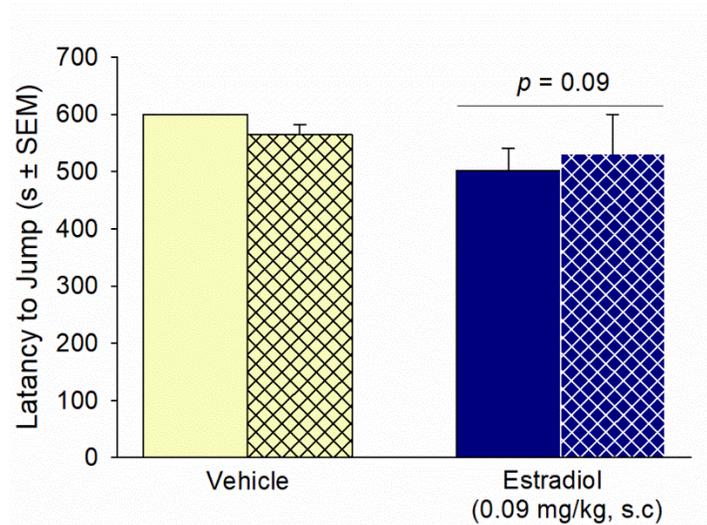

Figure 2B.   Latency to jump in vehicle and estradiol groups. Estradiol non-significantly reduced latency to jump ($p = 0.09$; n = 13/group).
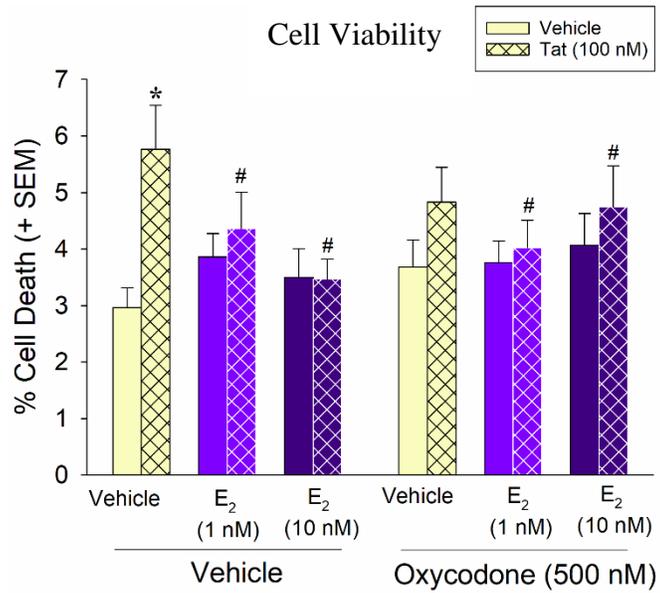
Figure 3A. Estradiol live/dead assay. Estradiol (E$_2$) significantly attenuated Tat-mediated neurotoxicity at 1nM ($p = 0.047$) and 10 nM concentrations ($p = 0.03$; n = 13-14).
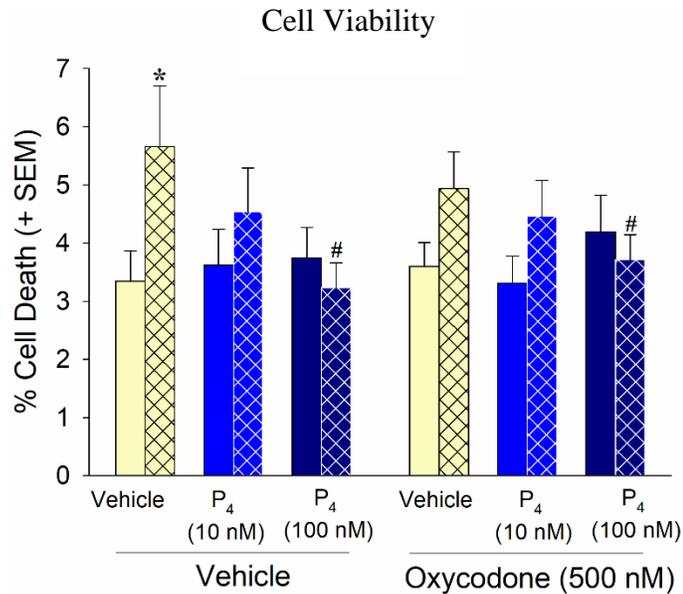

Figure 3B. Progesterone live/dead assay. Progesterone (P$_4$) significantly attenuated Tat-mediated cell death only at 100 nM concentration ($p = 0.003$; n = 17).