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Morphine efficacy is altered in conditional HIV-1 Tat transgenic mice

Sylvia Fitting^{1,*}, Krista L. Scoggins¹, Ruqiang Xu², Seth M. Dever¹, Pamela E. Knapp^{1,2}, William L. Dewey¹, and Kurt F. Hauser¹

¹Departments of Pharmacology & Toxicology, Virginia Commonwealth University, Medical College of Virginia Campus, Richmond, VA 23289

²Anatomy & Neurobiology, Virginia Commonwealth University, Medical College of Virginia Campus, Richmond, VA 23289

Abstract

Opiate abuse reportedly can exaggerate complications of human immunodeficiency virus type-1 (HIV-1) infection in the central nervous system (CNS), while opiate drugs are often indicated in the treatment of HIV-1-related neuropathic pain. Despite this quandary, few studies have assessed the relationship between the duration or extent of HIV-1 infection and the intrinsic neurobehavioral responsiveness to opioids. To address this problem, doxycycline (DOX)-inducible HIV-Tat₁₋₈₆ transgenic mice were used as a model for HIV-1-associated neurocognitive disorders, which permitted the regulation of Tat exposure and duration. The effects of continuous Tat induction on the activity of morphine were examined at weekly intervals using standard behavioral assays for nociception and motor function. In the spinal cord, Tat mRNA levels did not increase until the second and third weeks following induction, which corresponded to a significant loss of morphine antinociception as assessed in the tail-flick test. Alternatively, in the striatum, sustained increases in Tat mRNA expression during the second week of induction coincided with significant decreases in rotarod performance and interactions with morphine. Importantly, the behavioral effects of morphine differed depending on the timing and location of Tat expression; with increases in Tat transcript levels in the spinal cord and striatum corresponding to significant alterations in morphine-dependent nociception and rotarod performance, respectively. Assuming Tat levels contribute to the clinical manifestations of HIV-1, the results suggest that regional differences in viral load and opioid phenotype might influence the nature and degree that opiate responsiveness is altered in HIV-1 infected individuals.

Keywords

Neuro-acquired immunodeficiency syndrome (neuroAIDS); opioid drug abuse; nociception; spinal cord; striatum

*Corresponding Author: Sylvia Fitting, Ph.D., Dept. Pharmacology and Toxicology Virginia Commonwealth University Richmond, VA 23298 804-628-7579 (phone) 804-827-9974 (FAX) sfitting@vcu.edu.

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1. Introduction

The neuropathology, HIV-associated neurocognitive disorders, and distal symmetric polyneuropathy associated with HIV-1 infection have all been reported to be exacerbated by opiate drug use (Anthony et al., 2008; Anthony et al., 2005; Bell et al., 2006; Bell et al., 1998; Byrd et al., 2011; Robinson-Papp et al., 2012). Moreover, polymorphisms in the μ -opioid receptor (MOR) gene (OPRM1) have been suggested to alter the severity of HIV-1 infection and individual responsiveness to combination antiretroviral therapy (Proudnikov et al., 2012). The increases in HIV-1 pathogenesis caused by opioid abuse have largely been attributed to opioid suppression of immune function (Adler et al., 1993; Carr and Serou, 1995; Peterson et al., 1998). However, we and others additionally showed that opiate drugs, per se, can directly exacerbate the neurotoxic effects of the HIV-1 proteins Tat and gp120 in vitro using isolated central nervous system (CNS) neural cells (El-Hage et al., 2005; Gurwell et al., 2001; Hu et al., 2005; Turchan-Cholewo et al., 2006). Moreover, more recent studies suggest that opiates may principally act in glia (astroglia and microglia), and to a lesser extent directly in neurons, to exacerbate the neurotoxic effects of HIV-1 proteins (Podhaizer et al., 2011; Zou et al., 2011). Cross-talk between opiate drug and HIV-1 co-exposed microglia and astroglia appears to exacerbate critical proinflammatory and excitotoxic events (El-Hage et al., 2008; El-Hage et al., 2005; El-Hage et al., 2006b; Hauser et al., 2007; Zou et al., 2011). Many of these opiate interactions are mediated through μ opioid receptors in glia (Zou et al., 2011). This is especially apparent in the striatum, which shows a far more pronounced morphine-HIV-1 protein-induced cytokine production (El-Hage et al., 2005) compared to the cerebral cortex or spinal cord (Fitting et al., 2010b).

HIV-1 does not directly infect neurons; rather, the virus infects microglia and macrophages, and to a lesser extent astrocytes, contributing to neurodegeneration in part via the release of toxic viral proteins such as Tat and gp120 (Barks et al., 1997; Brenneman et al., 1988; Jones et al., 1998; Kramer-Hammerle et al., 2005; Philippon et al., 1994; Sabatier et al., 1991; Toggas et al., 1994; Tornatore et al., 1994). Tat, the transactivating protein for retroviral replication (Cann et al., 1985; Li et al., 2010) is profoundly neurotoxic through multiple molecular targets (Maggirwar et al., 1999; Magnuson et al., 1995; Nath, 2002; Nath et al., 1999; Perry et al., 2010). The Tat “virotoxin” is produced very early after infection, and is necessary for viral expression, cell-to-cell virus transmission and disease progression (Ensoli et al., 1993; Ensoli et al., 2006; Lin et al., 2003; Magnuson et al., 1995). Tat and other viral proteins, such as gp120 and Vpr, are likely agents of the observed neuronal loss in the brains of acquired immune deficiency syndrome (AIDS) patients and have been reported in the brain tissue of patients with HIV-1-associated neurocognitive disorders (Del Valle et al., 2000; Jones et al., 2007; Jones et al., 2000).

While it is generally accepted that opioid exposure can modify HIV-1 neuropathogenesis, emerging evidence suggests that the virus may also intrinsically alter endogenous opioid peptides and receptors (collectively termed the endogenous opioid system). For example, in vivo exposure to HIV-1 Tat causes widespread alterations in brain expression of μ opioid receptors and opioid receptors, as well as proopiomelanocortin, proenkephalin, and prodynorphin peptide genes (Fitting et al., 2010a; Turchan-Cholewo et al., 2008). The persistence of HIV-1 viral proteins in the brain has been shown to increase the effects of opiates in a HIV-1 transgenic rat model by upregulating μ opioid receptors and altering their biological and physiological effects (Chang et al., 2007; Chang and Connaghan, 2012; Lashomb et al., 2009). Moreover, HIV-1 selectively downregulates μ opioid receptor 1A splice variant transcripts in human microglia (Dever et al., 2012). These observations all suggest that normal opioid signaling is potentially altered in HIV-1-infected individuals.

The goal of the present study was to assess the extent to which the amount and duration of HIV-1 Tat expression would alter the neurobehavioral response to opioids. We used doxycycline (DOX)-inducible HIV-Tat₁₋₈₆ transgenic mice to test the hypothesis that HIV-1 Tat intrinsically affects the response of the CNS to opioids and that the effects of morphine are directly affected by Tat expression levels and by the duration of Tat exposure. To address this hypothesis, we used standard behavioral tasks for nociception (tail flick, hot plate) (Dewey et al., 1970; Langerman et al., 1995) as well as gross motor function (rotarod) (Goodkin et al., 1997; Kress and Kraft, 2005), and examined the efficacy of morphine in the presence and absence of Tat transgene expression. The results suggest that Tat expression levels are critical determinants for behavioral interactions with morphine and that regional differences in viral load and opioid phenotype influence the nature and extent of opiate actions in HIV-1 infected individuals.

2. Material and Methods

2.1. Mouse models

2.1.1. DOX-Inducible, Brain-Specific HIV-Tat₁₋₈₆ Transgenic Mice—The doxycycline (DOX)-inducible, brain-specific HIV-Tat₁₋₈₆ transgenic mice were developed on a C57BL/6J hybrid background and are described in detail elsewhere (Bruce-Keller et al., 2008). Tat expression, which is under the control of a tetracycline responsive, glial fibrillary acidic protein (GFAP)-selective promoter, was induced with a specially formulated chow containing 6 mg/kg DOX (Harlan, Indianapolis, IN), fed to both the Tat(–) controls [Tat(–)/DOX] and the inducible Tat(+) mice [Tat(+)/DOX]. Animals (~2 months of age, ~20 g, males) were exposed to a light-dark cycle of 12 h and with free access to water and DOX-containing chow.

2.1.2. Swiss-Webster Mice—In a control experiment, Swiss-Webster (SW) mice (~2 months of age, ~25 g, males) were obtained from Harlan Laboratories (Indianapolis, IN, USA) to test for non-selective effects of DOX on antinociceptive assays. SW mice have been and are continuously used for behavioral studies, such as tail-immersion and hot plate tests (Dewey et al., 1970; Sim-Selley et al., 2007) and were therefore chosen as an additional control. Half of the mice (n = 6) received normal chow and half (n = 6) received DOX supplemented food for 3 weeks before testing. Mice were exposed to a light-dark cycle of 12 h and with free access to water and the specified food.

2.2. Morphine Administration

Morphine was injected subcutaneously with each group receiving either saline (control), 2, 4, or 8 mg/kg morphine sulfate (MS, 1 ml/kg). 20 min after injection of saline or morphine the animals were subjected to different behavioral tests as described below.

2.3. Detection and quantification of Tat mRNA expression

Tat transgenic mice were humanely euthanatized by cervical dislocation. The spinal cord and the striatum (including C1 – C2 as well as the lumbar region up to L5) were rapidly dissected, frozen in liquid nitrogen and stored at –80 °C until use. Total RNA was isolated using miRNeasy Mini Kit (Qiagen, Inc., Valencia, CA), and utilized to generate cDNA by reverse transcription using the High Capacity cDNA Reverse Transcription Kit (Applied Biosystems, Foster City, CA), followed by PCR amplification of Tat or -actin (serving as a control for normalization) mRNAs. PCR reactions were performed in a total volume of 25 µL containing SensiMix™ SYBR qPCR reagents (Bioline USA Inc., Tauton, MA) using a Corbett Rotor-Gene 6000 real time PCR system (Qiagen, Inc., Valencia, CA). Primers used for Tat: forward 5′-gccctggaagcatccaggaagtc-3′; reverse 5′-cgctcgtctctccgcttcttct-3′. Primers for -actin: forward 5′-cgtgaaaagatgaccagatcatg-3′; reverse 5′-

cgctccggagtcctacacaa-3'. PCR conditions for detecting Tat mRNAs consisted of an initial hold at 95 °C for 10 min, followed by 38 amplification cycles of 95 °C for 5 s, 58 °C for 10 s, 72 °C for 20 s. For detection of -actin mRNAs, PCR conditions consisted of an initial hold at 95 °C for 10 min, followed by 28 amplification cycles of 95 °C for 5 s, 55 °C for 10 s, 72 °C for 20 s. PCR reactions were monitored by the amplification curves and PCR products were collected from the reactions terminated during the stage of linear amplification and subsequently loaded onto a 2% agarose gel for electrophoresis. The agarose gels were stained by ethidium bromide, and images were taken using a Kodak Image Station 440 (Kodak). The intensity of PCR amplification bands specific to Tat or -actin mRNAs was calculated using the ImageJ software available on the NIH website (<http://rsb.info.nih.gov>). Relative expression levels of Tat mRNAs were finally quantified by the band intensity of Tat gene normalized against the β -actin gene.

2.4. Testing Procedure

2.4.1. Experimental Design—The experiments were performed between 10:00 and 15:00 h and mice were tested once a week during a three-week period. All animal procedures were approved by the Virginia Commonwealth University of Institutional Animal Care and Use Committee (IACUC) and are in keeping with AAALAC guidelines. One week before the start of testing, the standard mouse chow given to both Tat(–) and Tat(+) mice was replaced with the specially formulated DOX chow, except in the control experiment to assess the effects of DOX, where half the SW mice continued to receive normal (non-DOX supplemented) mouse chow. Body weight was recorded immediately before testing. Baseline responses for tail flick, and hot plate were measured before animals received an acute s.c. morphine or saline injection. After the injection, the various behavioral tests were conducted in the following sequence: 30 min waiting period, assessment of tail-flick response, 10 min waiting period, assessment of hot-plate response, 10 min waiting period, assessment of rotarod performance. Acute morphine/saline injections and behavioral testing (baseline as well as test performance) occurred once a week.

2.4.2. Warm-Water Tail-Flick Test—The warm-water tail-flick test was performed using a water bath with the temperature maintained at 56 ± 0.1 °C. The withdrawal latency after drug treatment was assessed at 20 min for morphine, with a 10-s maximal cut-off time imposed to prevent tissue damage. Antinociception was quantified as the percentage of maximal possible effect (%MPE), which was calculated as $\%MPE = [(test\ latency\ control\ latency)/(10\ control\ latency)^{-1}] \times 100$ (Harris and Pierson, 1964). The %MPE value was calculated for each mouse using at least six mice per each morphine dose and treatment condition.

2.4.3. Hot-Plate Test—The hot-plate test was performed as described by O'Callaghan and Holtzman (O'Callaghan and Holtzman, 1975). The mice were first placed on an IITC Life Science Model 39D hot plate set at 56 °C to obtain baseline response latencies before drug administration. The mice were observed for licking their hind limbs or jumping in response to the heat. Mice were tested again at 30 min after s.c. morphine administration. A 30-s maximal cut-off was employed in order to prevent tissue damage. Antinociception was quantified as the percentage of maximum possible effect (% MPE), which was calculated as: $\%MPE = [(test\ latency - control\ latency) (30 - control\ latency)^{-1}] \times 100$ (Harris and Pierson, 1964). The %MPE value was calculated for each mouse using at least six mice per each morphine dose and treatment condition.

2.4.4. Rotarod Assessment—An accelerated rotating rod test allowed us to evaluate coordination and motor skill acquisition (Rotamex-5; Columbus, OH, USA). Mice were placed on the rod without any training period and the rod accelerated from 1 to 40 rpm in 1.0

rpm steps per 15 s. The time the mice spent on the rod without looping and falling was recorded. The SW mice were not assessed in the rotarod as they were tested in a separate experiment just to evaluate changes in baseline nociception due to DOX administration.

2.5. Statistical Analysis

All data were subjected to statistical analyses using analysis of variance (ANOVA) followed by Bonferroni's post-hoc analyses if necessary to determine statistical significance. A nonlinear regression model was used by fitting a sigmoidal $E_{\max}$ model to the behavioral versus morphine dose data. All data are presented as mean $\pm$ standard error of the mean (S.E.M.).

3. Results

3.1. Detection of Tat mRNA expression over a three-week period

Tat mRNA expression was detected prior to DOX-induction of the transgene and at one-week intervals for three weeks to mimic the duration of behavioral experiments. The relative expression levels of the Tat gene in the spinal cord and striatum of uninduced Tat(+) control mice and Tat(+)/DOX mice are shown in Fig. 1. In both the spinal cord and the striatum, basal Tat mRNA expression was observed in Tat(+) mice that did not receive DOX, which supports previous findings reporting constitutive, low levels of Tat expression throughout life even without DOX exposure (Bruce-Keller et al., 2008; Fitting et al., 2010a). More importantly, DOX treatment significantly increased Tat mRNA expression levels in the spinal cord of Tat(+) mice [main effect of Tat: $F(3, 12) = 7.80$, $p < 0.01$; Fig. 1A], with post hoc analyses only revealing significance for the second ($p < 0.01$, 3.45-fold increase) and third ($p < 0.05$, 2.63-fold increase) week of DOX exposure. In contrast, DOX treatment significantly increased Tat mRNA expression levels in the striatum [main effect of Tat: $F(3, 12) = 20.52$, $p < 0.001$; Fig. 1B] throughout the entire three-week period of Tat induction with a significant sustained increase in Tat mRNA for Tat(+) mice receiving DOX compared to the Tat(+) mice that did not receive DOX ($p < 0.01$ for all three weeks; week 1: 2.10-fold increase, week 2: 1.67-fold increase, week 3: 2.07-fold increase). These results suggest that the increases in Tat mRNA expression levels in the spinal cord were delayed compared to those in the striatum.

3.2. Warm-Water Tail-Flick Test

The acute antinociceptive effects of morphine were determined using the 56 °C warm water tail-flick test, which assesses predominantly a spinal reflex response (Langerman et al., 1995). Acute morphine injections increased the latency of tail withdrawal for Tat(-)/DOX and Tat(+)/DOX mice [main effect of morphine: $F(3, 82) = 73.87$, $p < 0.001$] but was significantly altered by Tat induction [Tat $\times$ morphine: $F(3, 82) = 2.95$, $p < 0.05$, Fig. 2A-C]. Tat(+)/DOX mice demonstrated a significant decrease in the efficacy of morphine as the experiment progressed [main effect of time: $F(2, 82) = 11.07$, $p < 0.001$; time $\times$ morphine: $F(6, 82) = 3.95$, $p < 0.01$], whereas no decrease in the efficacy of morphine was noted in Tat(-)/DOX mice (no significant time effect or time $\times$ morphine interaction). The greatest difference in tail flick latency was evident at the highest dose of morphine, with the efficacy of morphine being significantly reduced by the induction of the Tat transgene ($p < 0.05$). The loss in morphine efficacy was not seen during the first week of testing, but was increasingly evident over the three-week period [time $\times$ morphine: $F(2, 164) = 3.68$, $p < 0.01$]. Thus, Tat(+)/DOX mice were becoming less sensitive to the antinociceptive effects of morphine with increasing durations of DOX treatment and Tat induction. Importantly, no significant Tat induction effect was noted for the baseline tail-flick data, that is the baseline tail-flick response in the absence of morphine was unchanged during the three-week period. Tat(-)/DOX controls vs. Tat(+)/DOX was 2.66 ± 0.07 vs. 2.68 ± 0.10 in week 1, 2.22 ± 0.09

vs. 2.20 ± 0.06 in week 2, and 2.31 ± 0.08 vs. 2.32 ± 0.07 in week 3. It should be noted that for both groups morphine's effect decreased over the three week period, specifically for the 4mg/kg dose from week 1 to week 2 ($p < 0.05$). In a test to determine whether DOX itself might affect the tail-flick response, we used SW mice in which this response has been extremely well characterized. SW mice showed no differences in reaction time $\pm$ DOX supplemented food, indicating that DOX per se had no significant effect on withdrawal latency (Fig. 2D). SW mice displayed the same antinociceptive response to morphine [$F(3, 40) = 10.95$, $p < 0.001$] that we had previously observed in the Tat(-)/DOX control mice.

3.3. Hot-Plate Test

Acute morphine antinociception was further assessed in a task with more prominent supra-spinal components, using the same cohorts of mice. As for the tail-flick test, acute morphine injections increased the response latency to the hot-plate test in all three weeks (Fig. 3A-C) [main effect of morphine: $F(3, 82) = 105.02$, $p < 0.001$]. However, no difference was noted between Tat(-)/DOX controls and Tat(+)/DOX mice, indicating that the antinociceptive response to morphine was unaffected by Tat induction. The baseline response time remained unchanged throughout the experiment. Latency of the hot-plate response for Tat(-)/DOX controls versus Tat(+)/DOX mice was 7.57 ± 0.53 vs. 8.04 ± 0.43 in week 1, 9.59 ± 0.58 vs. 8.98 ± 0.46 in week 2, and 9.33 ± 0.63 vs. 9.17 ± 0.54 in week 3. The assessment of the hot-plate response in the SW mice indicates that DOX itself had no significant effect (Fig. 3D), with only a significant morphine effect [main effect of morphine: $F(3, 39) = 15.46$, $p < 0.001$].

3.4. Rotarod

Both Tat(-)/DOX and Tat(+)/DOX mice showed significant improvements in rotarod performance over the three-week period [main effect of time: $F(2, 76) = 26.42$, $p < 0.001$] (Fig. 4). A significant main effect of Tat [$F(1, 38) = 9.63$, $p < 0.01$] indicated that Tat(-)/DOX mice stayed significantly longer on the rotarod compared to Tat(+)/DOX mice, which was particularly seen in week 1 ($p < 0.01$) and week 2 ($p < 0.01$). Specifically, in the absence of morphine, Tat(+)/DOX mice performed significantly worse (had decreased latencies) on the rotarod [$F(1, 38) = 9.63$, $p < 0.01$] compared to Tat(-)/DOX controls (week 1: $p < 0.01$ and week 2: $p < 0.01$). Further, a significant main effect of morphine was noted [$F(3, 38) = 3.63$, $p < 0.05$], with morphine increasing the latencies of mice staying on the rotarod specifically in week 2 ($p < 0.05$) and week 3 ($p < 0.05$). Interestingly, acute morphine caused an improvement in the ability of the Tat(+)/DOX mice to stay on the rotarod [main effect of morphine: $F(3, 19) = 4.27$, $p < 0.05$], which was not seen in Tat(-)/DOX controls (no significant morphine effect or time $\times$ morphine interaction).

4. Discussion

The present study used in vivo models to test the activity of morphine on nociceptive and motor function in the presence and absence of the Tat transgene over a 1 to 3 week period. Behavioral effects of morphine appeared to differ depending on the timing and location of Tat expression; with increases in Tat mRNA levels in the spinal cord and striatum coinciding with significant alterations in morphine-dependent nociception and rotarod performance, respectively.

As previously reported (Fitting et al., 2010a), Tat protein was constitutively present at low levels in Tat(+) mice even without DOX treatment. Prior studies demonstrated increases in Tat protein by 7 d after DOX induction (Fitting et al., 2010a) with significant increases in gliosis (Bruce-Keller et al., 2008). IL-6 and RANTES changes at 48 h following Tat induction indicated a rapid onset of Tat expression with corresponding changes in CNS

pathology (Fitting et al., 2010a). Importantly, the present study demonstrates that in this transgenic mouse model, although Tat mRNA levels are increased throughout the CNS by DOX induction, Tat mRNA expression levels depend on the CNS region involved. Whereas the striatum showed uniform and sustained Tat mRNA expression levels during the three-week period of Tat induction, the spinal cord data indicated a slower course of induction, with increased Tat mRNA expression levels detected only after two and three weeks. Interestingly, when testing the efficacy of morphine in standard behavioral tasks associated with particular CNS regions, changes in the response to morphine appeared to coincide with Tat mRNA expression levels in those regions. For example, the tail-flick test, which largely involves spinal reflexes, showed a loss in morphine antinociceptive effects that paralleled the finding of significant Tat mRNA induction in the spinal cord. Alternatively, in a behavioral test assessing more striatal-associated functions, i.e. the rotarod, a marked deficit in performance was observed during the first and second week of testing, which coincided with more rapid increases in Tat levels in the striatum during these times.

The warm-water tail-flick and hot-plate assays are advantageous for testing nociception (Cochin, 1968). We consider the Tat(−) mice as the most valid control for their Tat(+) counterparts in terms of testing the effects of Tat induction by DOX, as both were developed on a C57BL/6J hybrid background and the only difference is that the control Tat(−) mice do not express the Tat gene. To determine whether DOX treatment might change the baseline nociceptive response, we chose SW mice as our test strain instead of comparing Tat(−) mice ± DOX. SW is a strain that has been commonly used to assess the activity of morphine on nociceptive tests (Dewey et al., 1970; Sim-Selley et al., 2007) and any DOX effects would have been easily detected in this strain. Importantly, no DOX effects were noted in the tail flick and hot plate. Morphine dose-dependent increases in antinociceptive responses were noted for the transgenic mice as well as the SW mice in both the tail-flick and the hot-plate tests. However, only the tail-flick test showed a loss in morphine's antinociceptive effects with the duration of Tat induction, thus coinciding with the Tat mRNA expression levels assessed in the spinal cord. Each test assesses a specific group of nociceptors or involves particular sites of the CNS, predominantly spinal vs. supra-spinal sites, in nociceptive processes. Therefore, dissimilarities between results due to different testing methods may occur. It has been shown that opiates act directly on spinal opiate receptors with spinal receptor activation to inhibit ascending signal transmission (Porreca et al., 1987). In contrast, for the hot-plate test morphine's antinociceptive effects were not altered by Tat induction. Even though the hot-plate does integrate an important component of the spinal reflex, previous literature has shown that the hot-plate assay predominantly measures a supraspinal response (Pertovaara and Hamalainen, 1994; South and Smith, 1998) where opioids activate interneurons that produce descending inhibition (Langerman et al., 1995). This is likely caused by cerebral/cortical inhibition, which we did not measure. Regional differences in Tat-induced cytokine/chemokine production (El-Hage et al., 2008; Fitting et al., 2010b) may be an additional reason for the differential response between tests. Cytokine production appears to be very much elevated in the spinal cord, cortex, and striatum compared to the cerebellum, particularly for MCP-1, IL-6, and TNF- α (El-Hage et al., 2008; Fitting et al., 2010b). The model of acute nociception has been studied before to assess the pharmacological profile of different opioid drugs in the presence of HIV-1 proteins (Chang and Vigorito, 2006; Chen et al., 2011; Palma et al., 2011). A recent study using the HIV-1 transgenic rat model reported an increased potency of morphine's antinociceptive properties for the HIV-1 transgenic rats with significantly lower ED₅₀ values for the HIV-1 transgenic rats compared to the F344 animals (Chang and Vigorito, 2006). In contrast, the HIV-1 protein gp120 has been shown to attenuate morphine-induced antinociception in a tail-flick assay (Chen et al., 2011), paralleling our findings in the present study that indicated a loss in morphine's antinociceptive effects in the Tat(+)/DOX animals. This finding is quite important as it suggests a change in the ability of HIV-1 infected patients to respond to

opioid treatments (Kimball and McCormick, 1996; Koeppe et al., 2010). Altered opioid/morphine sensitivity in HIV-1-infected patients would not be surprising, due to the fact that variable drug absorption in patients with advanced HIV-1 infection as well as an increased metabolic rate have been reported (Lefkowitz and Grant, 2005). Recent studies have reported that HIV-1-infected patients on opiates continued to experience significantly more pain than other patients or in other words no association between analgesic use and decreasing pain (Koeppe et al., 2010; Koeppe et al., 2012). If opiates exhibit altered nociceptive actions in the setting of HIV-1 infection, treatment choices need to be reconsidered but it should be noted that not all opiates may have the same effect. For example, a recent study showed that buprenorphine-induced antinociception was unaffected by gp120, whereas gp120 significantly diminished morphine- and methadone-induced antinociception (Palma et al., 2011).

The basal ganglia have been reported to be a major site of HIV-1 infection (Berger and Nath, 1997) and target for substance abuse (Hauser et al., 2005; Nath et al., 2000). The specific vulnerability of the basal ganglia to HIV-1 infection may result from phenotypic characteristics that are unique to striatal neurons and the selective vulnerability of dopaminergic afferent projections from the substantia nigra and ventral tegmental area (Ferris et al., 2008; Gelman et al., 2006; Kumar et al., 2011; Nath et al., 2000; Theodore et al., 2007). To evaluate basal ganglia function, the integrity of motor coordination was determined in the rotarod test, designed to evaluate maximal motor performance (Shiotsuki et al., 2010). The link between motor activity and the striatum is commonly made, but it does include other brain structures, such as cerebellum or cortex. Nevertheless, the striatum is usually the structure that shows deficits when rotarod performance is impaired (Haelewyn et al., 2007; Rogers et al., 1997). Our data indicate that in the absence of morphine, Tat(+)/DOX mice had worse performance on the rotarod compared to Tat(-)/DOX controls (Fig. 4), paralleling the increased Tat mRNA expression levels in the striatum after a one week Tat induction by DOX treatment. Interestingly, acute morphine exposure caused an improvement in the ability of the Tat(+)/DOX mice to stay on the rotarod, which was not seen in Tat(-)/DOX controls. Morphine and other opioids are known to have effects on motor activity with increasing locomotor activity at higher doses (Saito, 1990). Our data suggests that a morphine-mediated enhancement of motor coordination is specifically seen in chronic Tat exposed animals. It is well known that Tat increases inflammation (Nath et al., 1999), which has been reported in previous studies in regard to HIV-1 infection (Brack-Werner, 1999; Gorry et al., 2003; Patton et al., 1996; Vitkovic and da Cunha, 1995; Wang et al., 2004), and it is thought that morphine might exacerbate Tat-induced inflammation and worsen performance. Induction of pro-inflammatory mediators by Tat, such as time-dependent elevated production of CCL2/MCP-1, CCL5/RANTES and IL-6 by astrocytes, has been reported in multiple papers from our laboratory (El-Hage et al., 2005; El-Hage et al., 2006a; El-Hage et al., 2006b). Alternatively, it has been demonstrated that G-protein coupled opioid and chemokine HIV-1 co-receptors undergo heterologous, bidirectional cross-desensitization, including CCR5 and μ opioid receptor (Rogers and Peterson, 2003). Even though morphine has been demonstrated to potentiate the effects of Tat on CCL2/MCP-1 levels (Fitting et al., 2010a), other studies have shown that morphine attenuates the long-term consequences of inflammation (Laprairie et al., 2008). Improved rotarod performance by morphine has also been demonstrated in a bone cancer pain mouse model (Sarantopoulos, 2007). It should be noted that other factors may contribute to the differences seen in our transgenic mice on the rotarod, such as novelty, anxiety, and/or stress. For example, chronically stressed rats have been shown to display diminished performance on the rotarod (Mizoguchi et al., 2003) and the Tat(+)/DOX mice showed increased anxiety compared to Tat(-)/DOX controls in a light-dark choice test (unpublished observation). Our findings suggest that selective morphine and Tat-mediated behavioral interactions can

influence nociception and motor performance, and that the stage of HIV-1 infection may have a pronounced impact on opiate drug responsiveness.

5. Conclusion

Collectively, our findings demonstrate that Tat mRNA expression levels can modify the efficacy of morphine's effects on behavioral measures, such as antinociception and motor coordination/sensorimotor activity. We therefore speculate that regional differences in HIV-1 viral load, which leads to differential expression of viral proteins, causes differences in the behavioral response to morphine and other opiates.

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Figure 1.

(A) For the spinal cord, Tat induction with DOX caused significant increases in Tat mRNA levels in Tat(+) mice compared to Tat(+) mice without DOX. However, significant higher Tat mRNA levels were only noted for the second and third week of DOX exposure. (B) For the striatum, Tat induction with DOX caused significant increases in Tat mRNA levels in Tat(+) mice compared to Tat(+) mice without DOX. For all three weeks, Tat(+)/DOX mice expressed higher Tat mRNA levels compared to the control Tat(+) mice without DOX.; * $p < 0.05$ vs. Tat(+) mice without DOX, DOX = doxycycline.

Figure 2.

(A-C) Effects of the Tat transgene, morphine, and DOX on the latency of tail flick from a thermal nociceptive stimulus. Morphine caused a significant dose-dependent increase in the latency of tail withdrawal in the first week in all mice ($p < 0.05$) that was markedly attenuated in Tat(+)/DOX mice following prolonged Tat induction (Tat effect: $^{\$}p < 0.05$). The effects of prolonged Tat induction were most pronounced at higher doses of morphine. Further, a time dependent decrease in potency for morphine in both groups was noted as the experiment progresses, specifically for the 4 mg/kg dose from week 1 to week 2 ($p < 0.05$). (D) In SW mice, tail-flick latency was unaffected by 3 weeks of DOX exposure, while morphine significantly slowed the rate of withdrawal ($p < 0.001$), which was similar to morphine responsiveness in the control and Tat transgenic mice., TF: tail flick, DOX = doxycycline, SW = Swiss-Webster.

Figure 3.

(A-C) Effects of the Tat transgene, morphine and DOX on hot-plate response latency. Acute morphine exposure significantly increased the latency of responding to the hot plate in both Tat(-)/DOX and Tat(+)/DOX mice in all three weeks ($p < 0.001$). No difference was noted between the Tat(-)/DOX and Tat(+)/DOX mice. It should be noted that the dose response curve shifts slightly to the right over the three week period. (D) In control SW mice, chronic DOX administration had no significant effect on the hot-plate latency or the efficacy of morphine's effect ($p < 0.001$); SW and Tat transgenic mice displayed similar responses to morphine., HP: hot plate, DOX = doxycycline, SW = Swiss-Webster.

Tat transgenic Study

Figure 4.

(A-C) The Tat transgene affects rotarod performance. During the three weeks, both Tat(-)/DOX and Tat(+)/DOX mice stayed longer on the rotarod ($p < 0.001$). However, Tat(+)/DOX mice remained on the rotarod for a significantly less time than Tat(-)/DOX controls, and this was especially apparent in the groups that did not receive morphine (Tat effect and a Tat $\times$ morphine interaction: $^{\$}p < 0.05$). Interestingly, morphine exposure significantly increased the amount of time the Tat(+)/DOX mice remained on the rotarod in week 2 ($p < 0.05$), while rotarod performance in the Tat(-)/DOX controls was unaffected by morphine in any of the three weeks., DOX = doxycycline.