Effects of chronic expression of the HIV-induced protein, transactivator of transcription (Tat), on circadian activity rhythms in mice with or without morphine

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Running head: Effects of chronic Tat expression on circadian rhythms
ABSTRACT

Patients with human immunodeficiency virus (HIV) infection exhibit changes in sleep patterns, motor disorders and cognitive dysfunction; these symptoms may be secondary to circadian rhythm abnormalities. Studies in mice have shown that intracerebral injection of an HIV protein, transactivator of transcription (Tat), alters the timing of circadian rhythms in a manner similar to light. Therefore, we tested the hypothesis that chronic Tat expression alters circadian rhythms, especially their entrainment to a light:dark (LD) cycle, by using transgenic mice in which Tat expression in the brain was induced via a doxycycline (DOX)-sensitive, glial fibrillary associated protein (GFAP)-restricted promoter. Because opiate substance abuse, which shares comorbidity with HIV infection, also disrupts sleep a final experiment assessed the effects of morphine exposure on circadian rhythms in wild type and Tat transgenic mice. Mice housed in cages equipped with running wheels were fed chow with or without DOX. Experiment 1 revealed a small but significant (P<0.05) difference between groups in the phase angle of entrainment and a 15% decrease in the wheel running in the DOX group (P<0.005). During exposure to constant darkness, DOX did not alter the endogenous period length of the circadian rhythm. Experiment 2 investigated the effect of DOX on circadian rhythms in wild type and Tat(+) mice during exposure to a normal or phase-shifted LD cycle, or morphine treatment without any change in the LD cycle. Tat induction significantly decreased wheel running but did not affect entrainment to the normal or shifted LD cycle. Morphine decreased wheel running without altering the phase angle of entrainment and the drug’s effects were independent of Tat induction. In conclusion, these findings suggest that chronic brain expression of Tat decreases locomotor activity and the amplitude of circadian rhythms, but does not affect photic entrainment or re-entrainment of the murine circadian pacemaker.
Key words: HIV-AIDS, morphine, opioid drug abuse, locomotor activity, HIV encephalitis
INTRODUCTION

Infection with human immunodeficiency virus (HIV) induces expression of many viral and/or cellular factors that are not intrinsically infectious but may be involved in mediating some of the neural and psychological symptoms associated with HIV-AIDS, including alterations in sleep architecture and quality, fatigue, movement and cognition. It has been postulated that these symptoms may be secondary to abnormalities in the circadian timing system (8), which is a major regulator of the timing of sleep (see (27) for review) and is also a factor that influences cognition (7, 9, 12). Abnormalities in circadian rhythms of activity, body temperature, hormone secretion and circulating immune cells have been reported in HIV-AIDS patients as well as in non-human primates infected with simian immunodeficiency virus (17, 18, 36).

Recently, one HIV-induced protein, transactivator of transcription (Tat), has been shown to directly affect the timing, i.e., phase, of the mammalian master circadian pacemaker located in the hypothalamic suprachiasmatic nucleus (SCN) (8). Tat is released extracellularly by HIV infected glial cells and macrophages in the brain. It may then directly interact with neurons or be transported anterogradely or retrogradely along neuronal pathways (4). It may also activate uninfected glial cells to release a variety of factors that may in turn alter neuronal function (38). Administration of Tat protein to the murine SCN region in vivo or in vitro mimics the effects of acute light exposure on the circadian timing system (8). For example, similar to light pulses, SCN administration of Tat in the early subjective night induce phase delays in locomotor activity rhythms, while administration in the mid- to late subjective night induces phase advances (8). These Tat-induced circadian phase shifts are mediated by activation of N-methyl-D-aspartate (NMDA) receptors (8), as are photic phase shifts (10, 11, 26). Thus, by stimulating glutamatergic neurotransmission, administration of Tat can acutely regulate the circadian timing
system in a rodent. This finding suggested that chronic exposure to Tat as a result of HIV infection may alter the expression of circadian rhythms in humans, especially entrainment of circadian rhythms to the light:dark cycle (8). Furthermore, because many findings in a variety of species show that disrupted or dysynchronous circadian rhythms are associated with increased incidence of cardiovascular disease, increased tumor growth, decreased responsiveness to chemotherapy, memory deficits, and decreased longevity (7, 13, 14, 19-21, 29, 32, 45), it is possible that chronic Tat expression in the brain might contribute to HIV morbidity or mortality.

Because Tat induces activation of glutamate receptors, which mediates the phase shifting effects of light on circadian rhythms, chronic Tat exposure may interfere with entrainment to a lighting cycle or even mimic the effect of exposure to constant light. During exposure to constant light, circadian rhythms typically “free-run”, such that they gradually lose synchrony with external clock time (33, 34). Also, constant light exposure lengthens the endogenous circadian period (τ) in nocturnal rodents (33, 34). In order to investigate the effects of chronic Tat exposure on circadian rhythms, we used transgenic mice in which the Tat promoter has been linked to the doxycycline (DOX)-inducible tetracycline promoter. We tested the following hypotheses: 1) chronic Tat expression interferes with entrainment to a light:dark cycle, 2) chronic Tat expression increases the endogenous period length during exposure to constant darkness, and 3) chronic Tat expression alters the rate of entrainment to a new lighting cycle. Furthermore, because opiate addiction can be associated with HIV infection, and because opiates can aggravate the pathophysiological effects of HIV in the CNS clinically (1, 2) and in experiential models of HIV encephalitis (16, 30), we also tested the effect of the chronic exposure to the opioid drug, morphine, on circadian rhythms in mice expressing Tat.
MATERIALS AND METHODS

Experimental animals and housing conditions

Male mice, C3-C57Bl/6 cross, 5-9 months old, were used for these studies. Transgenic mice possessed an HIV-1 tat gene whose expression was driven by a tetracycline-inducible promoter. These transgenic mice were generated as follows: A tetracycline (tet) "on" system was used for generation of inducible constructs. Tat(1-86; HIV-1 IIIB) was cloned downstream of a tet responsive element (TRE) in the pTREX vector (Clonetech, Mountain View, CA). Reverse tetracycline transactivator (RTTA) was first cloned in pEGFP vector (Clonetech) at the BamHI/EcoRI site and further subcloned in GFAP promoter-driven vector pGfaLac-1 at the BamHI/BglII site. The construction of these plasmids and their validation using in vitro systems is described in a previous publication from the founder’s lab (6). For creation of transgenic lines, plasmid vectors were selectively digested with restriction enzymes and intact regulatory regions with respective genes and poly A sites were gel extracted. The purified fragments were used for inoculation of eggs. The founder animals (C3H x C57BL/6J) for each construct were checked for respective genes by PCR and southern blotting. Each founder line was then inbred to produce mice homozygous for the selected genes. Nervous system-restricted, inducible transgenic mice were created by crossing mice expressing the Tat gene under the TRE with mice engineered to express a GFAP promoter driven RTTA. Pure transgenic lines were derived by crossing homologous lines containing both the GFAP-RTTA and TRE-Tat genes. Thus, the inducible Tat transgenic mice (Tat(+) mice) described in this manuscript expressed both GFAP-RTTA and TRE-Tat genes, while control Tat(-) mice expressed GFAP-RTTA but not the TRE-Tat gene. It should be noted that GFAP-immunoreactive cells are abundant in the SCN (28).
Before experimentation, the mice were group housed and exposed to an alternating 14-h light (L):10-h dark (D) cycle with lights on at 6 AM. During experiments, the mice were individually housed in cages (32 by 14 by 12 cm) equipped with running wheels (11 cm diameter) electronically interfaced with hardware and software for recording and analyzing circadian locomotor activity data (ClockLab, Actimetrics, Willmette, IL). The cages were enclosed in ventilated, light-tight compartments, with 12 L:12D photoperiod. The light was provided by green light source, because previous studies have shown that circadian timing system is most sensitive to green light (~500 nm) (40). Water and food, either normal mouse chow (NC) [Harlan] or chow formulated to contain 6 mg of DOX per gram (DOX) were available ad libitum. Cages, food and water were changed weekly in both studies. All animal procedures, which were consistent with AAALAC guidelines, were reviewed and approved by the University of Kentucky Institutional Animal Care and Use Committee (IACUC).

**Experiment 1. Effect of DOX ingestion in Tat transgenic mice on expression of circadian locomotor activity rhythms during exposure to a light:dark cycle or constant darkness.**

The mice were exposed initially to an alternating light:dark cycle (12L:12D) with lights on at 7 A.M. The mice were fed either normal chow or DOX chow (as described above, N=6 per group). The phase angle of entrainment (time of nocturnal activity onset relative to time of lights off), and the total amount of activity per week, were recorded for each animal. After four weeks, the mice were transferred to constant darkness by leaving the lights off after the onset of the dark phase. The mice remained in constant darkness for seven weeks. During this time, dim red light was used for monitoring the animals and changing cages, food and water. The amount of total wheel running activity per week was determined. Also, the endogenous period length was
calculated by periodogram analysis using Clock Lab software. Body weight was monitored weekly as a possible indicator of nonspecific deleterious effects of the DOX-containing chow. At the end of the study, the mice were anesthetized and perfused transcardially with 4% paraformaldehyde in neutral phosphate buffer. Brains were dissected, washed in PBS, and flash frozen in OCT compound.

To confirm that Tat was expressed within the SCN, frozen, 20-µm-thick sections were cut serially through the SCN in the coronal plane. Using a dissecting microscope at 25-40 x magnifications, the region of the SCN was microdissected from four adjacent frozen sections per mouse. The tissue was collected using sterile, RNase-free micropipette tips and transferred immediately to RNA extraction buffer. RNA was isolated from tissue sections using the Absolutely RNA® Nanoprep kit (Stratagene) following manufacturer’s instructions and the RNA samples were treated with RNase-free DNase to remove genomic DNA contaminant. Total RNA was then reverse transcribed into cDNA using random hexamer primers by the high capacity cDNA reverse transcription kit (Applied Biosystems) at 25 °C for 10 min and 37 °C for 2 h in 20 µl total volume. cDNA was used as template in PCR amplification to measure Tat expression using the following primers: forward, 5’-gcc ctg gaa gca tcc agg aag tc; reverse, 5’-cgt cgc tgt ctc cgc ttc ttc ct. PCR was performed using a Touchdown cycle program. The amplified products were analyzed on 2% agarose gels with ethidium bromide, and photographed using a Kodak Image Station 440 system.

Experiment 2. Effect of DOX ingestion in wild type or Tat transgenic mice on expression of circadian locomotor activity rhythms during exposure to a normal or shifted light:dark cycle, and after exposure to morphine
This experiment investigated whether chronic expression of Tat affects the rate of entrainment to a phase-shifted light:dark cycle, and whether chronic morphine treatment influences circadian rhythms. The second issue was considered because many HIV-infected patients are addicted to opiates. Morphine exposure has been demonstrated to re-set the phase of circadian rhythms in mice (25) and opiate addicts show altered sleep-wake patterns (31). Importantly, opiates in combination with HIV can exacerbate some of the CNS effects seen with HIV alone. Therefore, we anticipated that morphine and Tat induction might selectively interact to affect circadian activity. Lastly, this study included wild type mice because information from other on-going studies and the results of Experiment 1 indicated that the Tat transgenic mice exhibit low levels of Tat expression even in the absence of ingestion of DOX. Therefore, in order to evaluate any non-specific effects of DOX ingestion on circadian rhythms, both Tat(+) and Tat(-) mice were fed the DOX chow in this experiment.

Male mice were weighed at the beginning of the study and randomly assigned to one of four groups (N=6 each): 1) wild type mice fed DOX chow, 2) wild type mice fed normal chow, 3) Tat (+) mice fed DOX chow, or 4) Tat (+) mice fed normal chow. The average initial body weight was not significantly different among the groups. The mice were individually housed in cages equipped with running wheels and running activity was recorded continuously. Food and water were provided ad libitum. At weekly intervals, the mice were weighed and fresh cages and water bottles were provided.

During Phase 1, the mice were exposed to a light:dark cycle (12L:12D, lights on from 7 A.M. to 7 P.M.) for three weeks. The phase angle of entrainment (time of nocturnal activity onset relative to time of lights off) and the amount of running activity (wheel revolutions per day) were recorded and analyzed.
During Phase 2, the timing of the light:dark cycle was shifted by 6 h, such that lights were on from 1 AM to 1 PM. (Thus, on the day of the light:dark cycle change, the animals experienced only 6 h of darkness, instead of 12. This method of advancing the LD cycle has previously been used in studies of phase shifting in mice (35). The number of days required for re-entrainment to the shifted light:dark cycle and the phase angle of entrainment were determined, as was the amount of wheel running activity.

Phase 3 of the study involved administration to morphine to all of the mice. Under isofluorane anesthesia, sterile surgery was conducted to implant subcutaneously a timed-release, morphine-containing pellet, obtained from the National Institute on Drug Abuse (NIDA; Rockville, MD, U.S.A). The pellets, which released 5 mg of morphine per day, were replaced with fresh pellets after 5 days. The phase angle of entrainment and the amount of running were determined.

Statistical analysis

Differences between groups in phase angle, total wheel running activity, endogenous period length, or body weight were assessed with repeated measures two-way analysis of variance examining the main effects of treatment and time, and their interaction. In the case of significant P values (P<0.05), post hoc analysis was conducted using Bonferroni’s test.
RESULTS

Experiment 1. Effect of DOX ingestion in Tat transgenic mice on expression of circadian locomotor activity rhythms during exposure to a light:dark cycle or constant darkness

Previous studies detected robust expression of HIV-1 Tat mRNA in the cortex, striatum, and hippocampus of inducible Tat(+) transgenic mice, while Tat transcripts were not detected in wild type [Tat(-)] mice or in tissues lacking a GFAP promoter (5), including the spleen and liver (unpublished results). Nevertheless, to confirm that Tat was expressed in the region of the SCN following chronic DOX administration, SCN tissue was micro-dissected from frozen tissue sections, RNA extracted and Tat mRNA expression assessed by RT-PCR analysis as described in Methods. Evaluation of PCR band intensities confirmed the presence of Tat mRNA in the region of the SCN following chronic DOX exposure in Tat(+) mice, while no signal representing Tat mRNA was observed in the Tat(-) mice (Fig. 1). Interestingly, a modest but detectable level of expression of Tat mRNA in the SCN was observed in Tat(+) mice that were not administered DOX, although Tat expression in these mice was much lower than expression in DOX-induced Tat(+) mice (Fig. 1). These data are in agreement with a previous study, in which we found that for some outcome measures vehicle-treated Tat(+) mice without DOX showed intermediate levels of change (e.g., reactive astrogliosis) compared to Tat(-) mice or Tat(+) mice treated with DOX, suggesting the Tat transgene was inherently “leaky” (5).

During four weeks of exposure to a light:dark cycle, all of the mice in both the DOX-containing chow and normal chow groups exhibited normal entrainment to the light-dark cycle with nearly all of the wheel running activity confined to the dark phase (Fig. 2). There was a small but statistically significant (P<0.05) difference between the groups in the phase angle of entrainment, i.e., the relationship of the onset of nocturnal activity to the lighting cycle (Figure
3). However, this effect was most pronounced during the first week of the experiment, during which the DOX-fed mice began the nocturnal activity five minutes before lights-off, in contrast to twenty three minutes after lights-off, in the case of the normal chow-fed mice. Although the daily activity patterns (actograms) displayed by individuals in both groups were quite similar (Figure 2), the total amount of wheel running activity was significantly (P<0.005) lower in the DOX-fed group (Figure 4A). In both groups, the total amount of wheel running activity increased significantly (P<0.001) across the four weeks (Figure 4A).

During exposure to constant darkness, all of the mice continued to show circadian rhythms of wheel running activity (see actograms in Figure 2). The endogenous period length of the circadian activity rhythm was not affected by DOX treatment (P=0.859) although there was a significant effect of time (P<0.001) (data not shown). The amount of wheel running activity throughout the exposure to constant darkness was not significantly affected by treatment (P=0.220) or time (P=0.440) nor was there a significant interaction effect (P=0.715) (Figure 4B).

The average body weight for each experimental group was stable across the study but was slightly but significantly greater (P<0.05) in the DOX-chow group as compared to the normal chow group mice (Mean±S.E.M., DOX: 27.9±0.2 versus normal chow [NC]: 26.7±0.2).

**Experiment 2. Effect of DOX ingestion in wild type or Tat transgenic mice on expression of circadian locomotor activity rhythms during exposure to a normal or shifted light:dark cycle, and after exposure to morphine**

All mice were entrained to the normal light:dark cycle (Phase 1), and successfully re-entrained to the 6-h phase advanced light:dark cycle (Phase 2) (Fig. 5). During exposure to either lighting cycle, the phase angle of entrainment was not significantly affected by either
genotype or food or an interaction of these conditions (data not shown). Furthermore, the number of days (mean of 8 for all groups) required for stable re-entrainment after the phase advance in the lighting cycle was not significantly affected by genotype (P= 0.89) or food (P=0.96), nor was a significant interaction of these effects observed (P=0.89).

In contrast to the absence of effects on entrainment, there were significant main effects associated with the Tat transgene and/or its induction (DOX treatment) on running wheel activity \((P \leq 0.0021)\) when assessed using a repeated measures (within-subjects design), analysis of variance (ANOVA) and post hoc Duncan’s multiple comparisons test (Statistica Version 6; Statsoft Inc., Tulsa, OK) (Fig.6). Tat induction significantly decreased activity compared to wild type mice receiving control \((P < 0.001)\) or DOX treated feed \((P < 0.05)\), or Tat(+) mice receiving control feed \((P \leq 0.005)\) (Fig. 6). Tat induction caused marked reductions in activity compared to other groups throughout the experiment irrespective of whether a 12-h light: 12-h dark cycle was advanced (Fig. 5, “Light: Dark Advance”) or whether chronic morphine was administered (Fig. 5, “Morphine”).

Advancing the 12-h light: 12-h dark cycle significantly increased running activity in individual mice (Fig. 6). There was a significant main effect of increase in running activity in mice when the time of lights-on was advanced from 0700 h (EST) to 0100 h \((P < 0.001)\). When individual mice were compared before and after the 6-h advance of the light phase, however, only wild type mice receiving control feed showed marked increases in activity \((*P \leq 0.02\) vs. “Light: Dark Regular” activity prior to light cycle advance). Mice in all groups showed increases \((28.1-53.6\%)\), albeit not significant, in running activity after the “lights-on” period was advanced, which likely contributed to the highly significant main effect. Tat induction caused
marked reductions in running activity compared to wild type mice receiving control feed after advancing the lights-on period ($P < 0.05$).

Unlike advancing the light:dark cycle, which increased activity, continuous morphine exposure (5 mg/day, subcutaneous) for three weeks significantly reduced wheel running across all groups ($P < 0.005$) (Fig.6, “Morphine”). Marked reductions in all groups were noted when activity during the “Light: Dark Advance” period before morphine treatment was compared to activity after morphine exposure. Moreover, since lighting conditions remained identical before and after morphine treatment, morphine caused the reductions in activity. By contrast, when activity following morphine treatment was compared to baseline activity prior to advancing the light:dark cycle, only Tat(+) mice receiving DOX ($P < 0.01$) or wild type mice receiving DOX ($P < 0.005$) showed diminished wheel running. Combined morphine treatment and Tat induction caused the greatest reductions in activity compared to activity in all other groups prior to or during morphine exposure (**$P < 0.005$) with the important exception of wild type mice that were co-administered morphine and DOX.

In contrast with effects on activity levels, morphine treatment did not alter the phase angle of entrainment. It was also observed that morphine treatment led to large decreases in body weight and also unexpectedly high rates of mortality (40-50%) in all treatments groups.

**DISCUSSION**

Previous studies have indicated that patients infected with HIV, as well as in non-human primates infected with simian immunodeficiency virus, exhibit alterations in a variety of circadian rhythms, including those of circulating hormones, circulating immune cells, body temperature, activity, and sleep (3, 17, 18, 36, 37, 41, 44). Furthermore, experimental studies in
mice show that the HIV protein, Tat, when acutely administered directly to the SCN, induces changes in the phase of circadian rhythms that mimic those induced by light (8). These findings led us to hypothesize that chronic exposure to Tat in transgenic mice would alter the entrainment of circadian rhythms to a light:dark cycle, but our results yielded very little support for this hypothesis. In all of the mice in both experiments, wheel running activity occurred almost exclusively during the dark phase, as previously observed for mice and other nocturnal rodents, demonstrating that entrainment to the lighting cycle was basically normal in all of the experimental animals. Induction of Tat by DOX induced a small change in the time of onset of the nocturnal activity (i.e., the phase angle of entrainment) in the first experiment but this effect was not observed in the second experiment. Furthermore, following the 6-h advance in the timing of the light:dark cycle in the second experiment, the Tat(+) transgenic mice, fed either DOX or regular chow, re-entrained to the new lighting cycle as quickly as the wild type mice did, demonstrating that chronic expression of Tat did not attenuate the capacity for re-entrainment.

In contrast to entrainment, the amount of wheel running activity was significantly affected by Tat expression. In both studies, the total amount of running activity was decreased in the Tat(+) mice fed DOX chow. Furthermore, because the wheel running activity occurred almost exclusively at night, the amplitude of the circadian rhythm of wheel running was lower in this group, reminiscent of previous findings concerning circadian rhythms after HIV infection. Interestingly, in the absence of DOX, Tat(+) mice displayed an intermediate level of wheel running that was in between that observed for wild type mice or Tat(+) mice receiving DOX. Although not significantly different from wild type mice receiving DOX, the trend suggested that there is limited expression of the Tat transgene in the absence of DOX. Ongoing studies in Tat
transgenic mice suggest that this assumption is correct and that “leakiness” of the Tat transgene may be revealed in some outcome measures. For example, intermediate levels of reactive astrogliosis have been observed in Tat(+) mice in the absence of DOX compared to both Tat(-) mice or Tat(+) mice receiving DOX (5).

In HIV patients, circadian rhythms in circulating hormones often exhibit reduced amplitude, and in some cases, the daily variation no longer constitutes a statistically significant rhythm (3, 36, 37). Reduction in the amplitude of the circadian rhythms of body temperature rhythm and activity has also been observed in Rhesus monkeys infected with simian immunodeficiency virus (SIV) (17, 18). Furthermore, SIV-infected monkeys exhibited lower levels of motor activity (17, 18). Thus, if DOX induced Tat expression in the mice in the present study (as expected), then the current findings suggest that Tat expression may be involved in decreasing activity and circadian rhythm amplitude during infection with SIV or HIV.

During exposure to constant darkness, the DOX chow-fed mice, as well as the normal chow-fed mice, continued to exhibit circadian rhythms in locomotor activity. This finding indicates that DOX exposure, and Tat expression, do not interfere with the generation and expression of circadian (i.e., endogenously generated) rhythms. Furthermore, the endogenous circadian period of the locomotor activity rhythm was less than 24 h and was not different between the DOX-chow and normal chow groups. This finding shows that DOX did not affect this basic parameter of the circadian timing system, and also suggests that the DOX-fed mice were not responding to constant darkness as if it were constant light, which typically increases the endogenous period length of circadian rhythms in nocturnal rodents (33).

Similar to Tat expression, chronic morphine treatment did not disrupt circadian wheel running rhythms or significantly alter their entrainment to the light:dark cycle. In contrast,
previous studies show that chronic morphine exposure can alter circadian activity and the expression of the murine Period-1 (mPer1) circadian clock gene (43). In another study, acute administration of fentanyl, which is a more selective μ opioid receptor agonist than morphine with considerable abuse liability, altered the phase of circadian wheel running rhythms and reduced the phase shifting effect of light exposure (42). The reasons for the discrepancy are uncertain but likely to result from differences in the dosage regimen. In the present study, morphine was administered continuously via time-release, pelleted implant, while in the prior studies cited above both morphine and fentanyl were administered through multiple daily injections. The physiological and behavioral consequences of repeated injections (“on-off”—with relative periods of drug withdrawal) versus continuous (“steady-state”) drug exposures are profound and may underlie different findings (23, 24).

In the present study, chronic morphine administration reduced wheel running activity. As noted above, because running is mainly restricted to the nighttime, this finding suggests that morphine reduces the amplitude of the circadian wheel running rhythm. Our findings are similar to previous findings showing that chronic morphine decreases wheel running in rats (39) and it is well established that the effects of chronic morphine on locomotor activity in mice are dependent on dose, duration of exposure, and genotype (15, 22). The current findings also suggested that Tat induction and morphine can interact independently from the Tat transgene to limit running. This potentially confounding interaction involving a marked reduction in activity has not been observed in shorter-duration studies (≤ 10 days) of Tat-morphine interactions using the Tat transgenic model of HIV encephalitis (HIVE) and has not been described in other studies. Alternatively, few studies co-expose mice to chronic, high dosages of DOX (>11 weeks) and morphine (3 weeks), which may create unique problems related to drug disposition and
metabolism. For this reason, we remain cautious in our interpretations of Tat-morphine interactions in the present study.

Chronic induction of Tat did not induce weight loss or to any other indication of morbidity. In fact, the DOX-fed mice exhibited somewhat greater body weights than the control chow-fed mice in Experiment 1. Because initial body weights were not determined in this study, it is not certain if the body weight difference was due to the DOX or to an inadvertent assignment of heavier animals to this group, although DOX has not been shown to affect feeding in other studies and mice were arbitrarily assigned to experimental groups in our studies. It was interesting to note that the DOX-fed animals exhibited significantly less running activity, a factor that could contribute to higher body weights. In contrast with Experiment 1, the results of Experiment 2 did not indicate a significant effect of either Tat-expression or DOX exposure on body weight.

In conclusion, the present studies in mice suggest that chronic exposure to Tat decreases locomotor activity and the amplitude of the circadian locomotor activity rhythm but does not disrupt the expression of this rhythm during exposure to either a light:dark cycle or constant darkness. In contrast to previous demonstrations that acute administration of Tat to the mouse SCN at night mimics the circadian phase resetting effect of light (8), the present findings show that chronic exposure to Tat does not have any major effect on photic entrainment or re-entrainment of the murine circadian pacemaker. Furthermore, exposure to morphine concomitantly with Tat-expression had no major effect on the expression of circadian rhythms.
PERSPECTIVE AND SIGNIFICANCE

Infection with the HIV or SIV virus is associated with decreased locomotor activity and/or alterations in circadian rhythms, especially reduction in rhythm amplitude (3, 17, 18, 36, 37, 41, 44). Indeed, one longitudinal study of SIV infection demonstrated progressive decreases in circadian rhythm amplitude preceding ultimate loss of circadian rhythms (17, 18, 36). The present findings suggest that lower activity levels and reduced circadian rhythm amplitude in HIV-infected patients may be caused at least in part by chronic expression of the HIV-induced protein, Tat, in the brain. Concurrent exposure to chronic morphine further inhibits the amplitude of the activity rhythm suggesting some potential common mechanisms with HIV Tat. These findings add to a growing body of evidence that exposure to HIV proteins within discrete regions of the CNS mediates specific physiological and behavioral symptoms that are characteristic of HIV-AIDS patients.

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FIGURE LEGENDS

Figure 1. Inducible Tat expression in the SCN region. RNA was extracted from microdissected SCN tissue and analyzed by RT-PCR as described in Methods. Images depict the presence or absence of an amplification product with the predicted size of 141 bp in samples derived from the SCN of two Tat(-) mice and four Tat(+) mice, as well as positive and negative (non-template) control samples. The four Tat(+) mice included two that were fed DOX-chow (lanes 4 and 6), and two that received normal chow (lanes 3 and 5).

Figure 2. Representative activity records for Tat(+) mice. The activity records are double-plotted, such that the horizontal axis represents 48 consecutive hours, with clock times indicated on the top. The vertical axis represents successive 48-h periods, from top to bottom. The top line represents days 1 and 2, the next line represents days 2 and 3, the third line shows days 3 and 4, etc. Wheel running activity is indicated by the dark marks. The animals were exposed to a light:dark cycle (represented by the horizontal black and white bars shown at the top) for the first four weeks, and then were exposed to constant darkness (indicated in gray). Triangles represent times that cages were changed. NC, normal chow; DOX, doxycycline-containing chow.

Figure 3. Phase angle of entrainment to the lighting cycle in Tat(+) mice. Phase angle was calculated as the difference between the time of lights off and the time of nocturnal activity onset. Bars represent the mean ± S.E.M. NC, normal chow; DOX, doxycycline-containing chow. 2-Way ANOVA revealed a significant effect of treatment (P<0.02) but not time (P=0.83) and a significant interaction effect (P<0.05). *P<0.01, compared to normal chow fed mice at the same week, based on Bonferroni’s test.

Figure 4. Effect of doxycycline (DOX) treatment or normal chow (NC) on wheel running activity in Tat(+) mice. Bars represent the mean ± S.E.M. of total wheel running activity per
week. During exposure to a light:dark cycle (top panel), wheel running activity was significantly affected by DOX (P<0.005) and time (P<0.0001), but there was not a significant interaction effect (P=0.75). During exposure to constant darkness (bottom panel), total wheel running activity was not significantly affected by treatment or time.

**Figure 5.** Representative activity records for wild type (WT) and Tat(+) mice fed normal chow (NC) or doxycycline-treated chow (DOX). Mice were exposed to a regular light:dark cycle (represented by the horizontal black and white bars shown at the top) until the day indicated by the triangle on the left axis, when the light:dark cycle was advanced by 6 h (represented by the horizontal black and white bars shown at the bottom). The double triangles indicate the day of onset of morphine treatment. See Figure 1 for explanation of the activity records.

**Figure 6.** Total amount of wheel running activity in wild type and Tat(+) mice. Tat induction with doxycycline (DOX) led to significantly lower levels of running activity. (See Results section for further explanation of these data). * P<0.02 compared to the same group exposed to the regular light:dark cycle; ** P<0.005 compared to all other groups during or prior to morphine treatment; § P ≤ 0.023 compared to wild type mice receiving control feed after advancing the lights-on period.