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The HIV-1 associated protein, Tat_{1–86}, impairs dopamine transporters and interacts with cocaine to reduce nerve terminal function: a no-net-flux microdialysis study

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Abstract

Injection drug use accounts for approximately one-third of HIV-infections in the United States. HIV associated proteins have been shown to interact with various drugs of abuse to incite concerted neurotoxicity. One common area for their interaction is the nerve terminal, including dopamine transporter (DAT) systems. However, results regarding DAT function and regulation in HIV-infection, regardless of drug use, are mixed. Thus, the present experiments were designed to explicitly control Tat and cocaine administration in an *in vivo* model in order to reconcile differences that exist in the literature to date.

We examined Tat plus cocaine-induced alterations using no-net-flux microdialysis, which is sensitive to alterations in DAT function, in order to test the potential for DAT as an early mediator of HIV-induced oxidative stress and neurodegeneration *in vivo*. Within 5 hours of intra-accumbal administration of the HIV-associated protein, Tat, we noted a significant reduction in local DAT efficiency with little change in DA overflow/release dynamics. Further, at 48 hrs post-Tat administration, we demonstrated a concerted effect of the HIV-protein Tat with cocaine on both uptake and release function. Finally, we discuss the extent to which DAT dysfunction may be considered a predecessor to generalized nerve terminal dysfunction.

Characterization of DAT dysfunction *in vivo* may provide an early pharmacotherapeutic target, which in turn may prevent or attenuate downstream mediators of neurotoxicity (i.e., reactive species) to DA systems occurring in NeuroAIDS.

Keywords

HIV; Cocaine; Dopamine; Dopamine Transporter; Microdialysis; Tat

Human Immunodeficiency Virus (HIV) is the leading cause of dementia among individuals under the age of 40 (Mattson et al., 2005). HIV-associated neurological decline (HAND) most

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severely affects subcortical dopamine systems (Aylward et al 2003, 2005; Paul et al., 2002, 2005). Accordingly, HAND resembles other subcortical dementias like Parkinson's disease (Berger and Nath, 1997; Koutsilieri et al., 2002; Sporer et al, 2005). Injection drug use accounts for one-third of all HIV-infections in the United States (NIDA, 2004). Additionally, the HIV-infected, drug dependent population demonstrates poor adherence to HIV therapy, leading to the possibility of a variant of HAND that is uniquely expressed in this population. Indeed, the HIV-infected, drug dependent population (UNAIDS/WHO, 2007) may experience a more severe manifestation of HIV-neurobiological impairment. This is evidenced, independent of therapy, by demonstrations of concerted neurotoxicity associated with HIV-associated proteins plus (+) drugs of abuse like cocaine (Turchan et al., 2001; for review, see Ferris et al., 2008).

HIV proteins, like the transcriptional transactivator (Tat), are actively released by HIV-infected cells and can interact with neurons independent of HIV. The mechanism for concerted neurotoxicity of Tat + drugs of abuse is unknown. Nevertheless, it is now well-established that increased levels of reactive oxygen species (ROS) contribute to decreased neuronal viability in both HIV infection (Aksenov et al., 2001; Turchan et al., 2003) and in various substance abuse disorders (Imam et al, 2001; Quinton and Yamamoto, 2006). Following HIV-protein administration ROS may occur as a result of aberrant immune responses (Mollace et al., 1995, 2001; Floyd et al., 1999). For some abused substances like methamphetamine, aberrantly high and extended dopamine levels are thought to auto-oxidize and mediate the potentiation of ROS (Quinton and Yamamoto, 2006; Cadet et al., 2007).

Given that both methamphetamine and HIV-proteins increase ROS, it is clear they may interact in some concerted fashion to potentiate disruption of neuronal integrity. It is less clear, however, how other psychostimulants, like cocaine, interact with HIV-proteins to disrupt neuronal integrity. This lack of clarity stems from two sources: 1) cocaine and methamphetamine do not share the same interaction at the dopamine transporter (DAT), and 2) unlike methamphetamine, cocaine alone is not toxic to DA systems despite potentiation of Tat-induced free radical formation and toxicity. One recent theory suggests that a Tat-induced nerve terminal degradation and DAT dysfunction may reduce the capacity for DA to be properly recycled (Ferris et al., 2008). Thus, when paired with an agent that promotes release of DA from intracellular pools, such as cocaine (Venton et al., 2006), aberrant DA release and uptake kinetics may facilitate the increase in free radical production.

Indeed, recent research suggests the time-frame for HIV-protein induced DAT dysfunction precedes the timeframe for decreases in neuronal viability *in vitro* (Aksenova et al., 2006; Wallace et al, 2006). A reduction in DAT uptake occurs within 30 min. of Tat administration (Wallace et al., 2006), while a reduction in DAT binding has been demonstrated by 2 hrs (Aksenova et al., 2006). These time-points correspond to increased free radical production from a separate study (2 hrs), but the decrease in neuronal viability (24 – 48 hrs) does not occur until well after the timeframe for DAT dysfunction (Aksenov et al., 2003; Ferris et al., 2009). Thus, significant attenuation of DAT function likely occurs earlier than degeneration of nerve terminals. This contention is important in that it implies a Tat-induced reduction in uptake and release kinetics may facilitate nerve-terminal degradation, and thereby emerge as a therapeutic target for HIV-infected cocaine users. Nonetheless, to date, the DAT as a therapeutic target for HIV-infected drug abusers has received little attention. In addition, the work that has been accomplished has demonstrates mixed results. Some research has shown decreased DAT binding in HIV-infected patients (Wang et al., 2004; Chang et al., 2008), while postmortem analyses indicate an increase in DAT binding (Gelman et al., 2006). *In vitro* work has shown a Tat-induced decreased DAT binding with no change in DAT immunoreactivity (Aksenova et al., 2006).

Thus, the present experiments were designed to explicitly control Tat and cocaine administration in an *in vivo* model in order to reconcile differences that exist in the literature to date. Specifically, we will test 1) whether Tat-induced DAT dysfunction shown *in vitro* can be demonstrated in awake, freely-moving animals, 2) whether DAT dysfunction occurs earlier in time than a loss of nerve-terminal function as measured by capacity for significant increase in DA overflow, and 3) whether cocaine moderates any Tat-induced alteration in nerve terminal uptake and release capacity.

2. Experimental Procedures

No-Net-Flux microdialysis (NNF) is well suited-, and possesses high specificity-, to test DAT function *in vivo*, and when paired with conventional microdialysis within the same animals, can serve as a means to directly test the functional integrity of remaining nerve terminals to mediate the overflow of DA (Parsons et al., 1991; Smith and Justice, 1994; Tang et al., 2003). Cocaine is known to induce neuroplasticity to structures that govern release and uptake kinetics at the synapse (Kalivas and Duffy, 1993; for reviews, see Zahniser and Sorkin, 2004; Pierce and Kumaresan, 2006). Therefore, in an effort to model the effect of HIV-proteins on the DA system of cocaine users, and test any interaction between Tat and previous experience with cocaine, we repeatedly administered cocaine to animals for several days prior to administration of Tat. This injection schedule has been shown to induce neurochemical and behavioral sensitization associated with cocaine induced neuroplasticity (Ferris et al., 2007). Finally, we combined this model of drug use with an injection procedure of recombinant Tat which has repeatedly demonstrated a Tat-induced increase in neurotoxicity and oxidative stress using neuropathological and stereological techniques (Aksenov et al., 2001, 2003; Fitting et al., 2008).

2.1. Animals

Twenty-four male Sprague-Dawley rats (300 – 350 g; Harlan Laboratories, USA) were double-housed on a 12-hour light/dark cycle with food and water available *ad libitum*. All animals were handled daily for one week prior to surgery. Animals were maintained according to the National Institutes of Health (NIH) guidelines in Association for Assessment and Accreditation of Laboratory Animal Care (AAALAC)-accredited facilities. The experimental protocol was approved by the Institutional Animal Care and Use Committee at the University of South Carolina, Columbia (assurance number A-3049-01).

2.2. Surgery

All animals were anesthetized prior to surgery using sevoflurane gas, induced using 7% inhalant and maintained at 3% inhalant for the duration of the surgery. All animals received one microdialysis guide cannula (Bioanalytical Systems, Inc., USA) in either the left or the right ventromedial striatum (NAc; counterbalanced across group) using stereotaxic coordinates AP + 1.2 mm, L $\pm$ 2.0 mm, DV – 5.0 mm relative to Bregma, midline, and skull surface, respectively. Guide cannulas were fixed to the skull with skull screws and dental acrylic/cement. Following surgery, animals were given one subcutaneous injection of buprenorphine for pain relief (0.1 mg/kg).

2.3. Injection and Microdialysis Schedule

The experiment consisted of 11 days of once-daily injections of either cocaine (Coc, 10 mg/kg/ml) or saline (Sal, 1 ml/kg) and 2 microdialysis sessions (on days 9 and 11). The injection and microdialysis schedule is described herein; specifics of the microdialysis sessions are described in subsequent sections.

On the morning after surgery (Day 1), all animals were randomly assigned to either the Sal or Coc treatment group. All animals then received one daily intraperitoneal (i.p.) injection of either Sal (1 ml/kg) or Coc (10mg/kg/ml) in their home cages for 8 consecutive days (i.e., Days 1 – 8) and on the day between microdialysis sessions (Day 10).

Immediately preceding the first NNF microdialysis session (Day 9), awake freely-moving rats were administered an intra-accumbal infusion of the recombinant HIV-1 protein Tat₁₋₈₆ (Diatheva, Italy), or vehicle (Veh, aCSF) control, depending on randomly assigned group membership; splitting the Sal and Coc treated groups into 4 groups (Veh+Sal, Veh+Coc, Tat+Sal, Tat+Coc). Following the completion of NNF procedures, animals received their respective Coc or Sal injection in the microdialysis chambers in order to monitor their neurochemical response to Coc or Sal.

Two days (Day11) following the first microdialysis session and micro-infusion of Veh or Tat₁₋₈₆, a second NNF microdialysis session was performed. Following the completion of the NNF procedures, all animals received a final challenge injection of Coc (10 mg/kg/ml) to monitor their neurochemical response Coc challenge. On the day following the last microdialysis session, animals were sacrificed and brains were extracted and frozen for histological assessment of probe placement.

2.4. Microdialysis (in vivo)

The procedure for dialysis during Session 1 (day 9) and Session 2 (day 11) was identical. On the morning of each microdialysis session, stylets were removed from the guide cannulas and replaced with a microdialysis probe. This probe possesses a semipermeable polyacrylonitrile membrane extending 2.0 mm beyond the ventral tip of the guide cannulas. Prior to the start of NNF procedures, the probes were perfused at 1.0 µl/min with artificial cerebrospinal fluid (aCSF; pH 6.5) composed of: NaCl 150 mM, KCl 3.0 mM, CaCl₂ 1.7 mM, MgCl₂ 0.9 mM, D-glucose 4.9 mM, and ascorbic acid.25 mM.

NNF procedures began 2 h and 15 min following probe insertion. For NNF, multiple concentrations of DA in aCSF (0, 5, 10, 20 nM) were perfused through the inlet line of each animal for a 45 min equilibration period plus two 15 min fractions. The four DA concentrations were administered according to a latin-square design that was further counterbalanced to account for immediate sequential order effects (Bradley, 1958). This design assured equal representation of every order across groups; and precluded immediate sequential order effects by ensuring each concentration followed each of the other concentrations equally as often.

Once all four concentrations had been perfused for each animal to conclude the NNF procedure, the inlet line and probe for every animal was perfused for a 45 min equilibration period with aCSF containing no DA or ascorbic acid, followed by collection of dialysates at 1.0 µl/min for 15 min fractions. After the third baseline collection, animals were given an i.p. injection of either Coc (10 mg/kg/ml) or Sal (1 ml/kg) within the testing bowls according to group membership established on Day 1. During Session 2, all animals received an i.p. injection of Coc (10 mg/kg/ml) at this time-point. Both sessions ended after collecting five fractions following the injection. Dialysates in both the NNF and standard dialysis procedures were collected in vials with 5.0 µL of a solution containing perchloric acid (0.05 N), sodium bisulfite (200 mM) and EDTA (1.0 mM) to minimize spontaneous oxidation of DA.

2.6. Microdialysis (in vitro)

The day following both Session 1 and Session 2, six probes that had been used for *in vivo* sampling the day before were randomly chosen for *in vitro* calibration procedures. Each probe was suspended in a beaker filled with continuously well-stirred aCSF (see recipe above)

containing no DA and warmed to body temperature (37°C). The probes were perfused with aCSF containing 0, 5, 10, and 20 nM DA for a 15 min equilibration period plus a single 15 minute fraction per concentration at 1.0 µl/min. The fractions were quantified to determine *in vitro* recoveries.

2.6. HPLC and Data Analyses

All dialysates were analyzed by liquid chromatography with electrochemical detection. Separation of DA from metabolites was achieved by injecting 15 µl of each sample onto a C-18 analytical column (100 × 1 mm; 3µm; BAS) using a mobile phase (pH 6.0) containing 1 L HPLC grade H₂O, 60 mM Sodium Acetate, 0.50 mM disodium EDTA, 0.50 g 1-octane sulfonic acid, and 15:100 ratio of acetonitrile to HPLC H₂O at a flow rate of 100 µl/min. DA was detected by oxidation at a glassy carbon electrode with an applied potential of +650 mV versus an Ag/AgCl reference electrode. To quantify dialysate DA concentrations, the 5, 10, and 20 nM DA solutions used in the dialysis procedure were directly injected on to the HPLC and used to form the standard linear curve; maintaining goodness-of-fit values of $R^2 = 0.99$.

For NNF procedures, the two dialysates collected for each concentration were averaged, giving the concentration of DA that exited the probe (C_{out}), and this concentration was subtracted from the corresponding concentration perfused into the probe (C_{in}), providing a difference score or an index of gain or loss of DA at each concentration ($C_{in} - C_{out}$). First order regression was then used to determine the best fit lines for each group, regressing $C_{in} - C_{out}$ on C_{in} (Parsons et al., 1991; Smith and Justice, 1994; Tang et al., 2003). The slope of the regression line provides the extraction efficiency (E_d), which has been shown to be a reliable measure of DAT function (Smith and Justice, 1994). The x-intercepts when $y = 0$ provides the extracellular DA levels (DA_{ext}). The degree to which NNF microdialysis predicts *absolute* extracellular DA concentrations in the striatum remains unresolved, as levels are highly variable across studies (cf, Peters and Michael, 1998). Thus, we took the approach suggested by Westerink (1995) and discuss our results in terms of treatment-induced changes relative to control; making no interpretation concerning absolute DA levels.

For within-session analyses, the difference scores were used as dependent variables in 4 (concentration; within) × 2 (Tat vs. Veh) × 2 (Coc vs. Sal) mixed ANOVA. A Concentration X Tat or Coc interaction indicates a significant difference between the E_d when collapsing Coc or Tat, respectively. Additionally, the slopes and x-intercepts when $y = 0$ were used as dependent variables in planned comparisons in order to further characterize differences in these parameters between the four groups.

For the conventional dialysis procedures, basal DA efflux for each animal in each session was defined as the average of the baseline values. All data were converted to percent of baseline to be used as the dependent measure.

3. Results

Two animals, distributed across different treatment groups, were lost on the day between sessions 1 and 2 due to complications with the head-mount. Histological analysis confirmed that all probe tracts were centered in the nucleus accumbens core, with some degree of extension into the ventromedial striatum or lateral accumbens (Figure 1).

3.1. Effect of Tat and cocaine on DA_{ext} and E_d within 5 hrs of administration

NNF microdialysis during this session was completed within 5 hrs of Tat-administration, and demonstrated a significant reduction in E_d for Tat treated animals, regardless of previous experience with Coc. E_d has been widely accepted as an indirect measure of DAT function

(Smith and Justice, 1994), suggesting Tat reduced DAT function within 5 hrs of administration. DA_{ext} was statistically no different for any group; and in the context of decreased uptake, this result suggests degenerating nerve terminals (see Discussion).

With respect to E_d , a 4 (concentration; within) $\times$ 2 (Tat vs. Veh) $\times$ 2 (Coc vs. Sal) mixed ANOVA using gain/loss scores as dependent variables indicated a main effect of Tat, $F(1, 18) = 30.96$, $p < .0001$, and Concentration, $F(3, 54) = 431.67$, $p < .0001$. Furthermore, there was a Concentration X Tat Interaction, $F(3, 54) = 12.59$, $p < .0001$, but no Concentration X Coc or Concentration X Tat X Coc three-way interaction, indicating that Tat treatment, but not Coc, altered the E_d (Figure 2). The interaction in this ANOVA is essentially the same as a main effect of Tat when using slope as the dependent variable, $F(1, 24) = 30.95$, $p < .001$. Planned comparisons were utilized to further characterize and validate this analysis. Between subjects t-tests using slope as a dependent variable verified that regardless of whether animals received Coc or Sal, Tat significantly altered E_d ; $t(10) = 2.81$, $p < .01$ for Coc-treated animals, and $t(8) = 4.56$, $p < .01$ for Sal-treated animals (Figure 2). Again, no effect of Coc on E_d was present regardless of whether the animals received Tat or Veh. Independent samples t-tests using slope as a dependent variable indicated no difference between *in vitro* probe recovery and *in vivo* Veh+Sal and Veh+Coc, but a significant difference when comparing *in vitro* to *in vivo* Tat +Sal, $t(9) = 5.080$, $p < .01$, and Tat+Coc, $t(10) = 3.448$, $p < .01$. With respect to DA_{ext} , a 2 X 2 ANOVA using intercept values as the dependent measure indicated that there was no main effect of Tat, Coc, or a Tat X Coc interaction.

3.2. Effect of Tat on cocaine-induced DA overflow dynamics within 5 hrs of administration

Conventional microdialysis following NNF procedures demonstrated that Coc significantly elevated DA levels in both Tat- and Veh-treated animals. DA overflow for Tat and Veh-treated animals was not significantly different. An 8 (Time, within) X 2 (Tat vs. Veh) X 2 (Coc vs. Sal) ANOVA indicated a main effect of Coc, $F(1, 13) = 10.86$, $p < .01$, and interactions of Time X Tat, $F(7, 91) = 2.76$, $p < .05$ and Time X Coc, $F(7, 91) = 6.07$, $p < .0001$. The 8 X 2 ANOVA includes the 3 baseline fractions in the analysis. Thus, specific relationships between each group's responses to injection were analyzed with a 2X2 ANOVA using area under the response curve (AUC) as the dependent variable, demonstrated in Figures 3A and 3B. The 2 X 2 ANOVA upheld a main effect of Coc $F(1,15) = 12.31$, $p < .01$, but no main effect of Tat or Tat X Coc interaction. Since the main effect of Coc collapses the Tat and Veh treated animals, planned comparisons indicated that the main effect of Coc was upheld when examining both groups individually (Figure 3A and 3B), indicating that Tat has not occluded Coc's efficacy by Session 1 despite reduced DAT efficiency demonstrated in NNF procedures.

3.3. Effect of Tat and cocaine on DA_{ext} and E_d 48 hrs after administration

NNF microdialysis during this session was completed 48 hrs after Tat-administration. In this session, Coc potentiated the Tat-induced reduction in E_d while preventing compensatory increases in DA_{ext} following Tat-induced terminal degeneration. This result suggests that Coc facilitated degeneration of nerve-terminals 48 hrs after Tat-administration, effectively reducing the both uptake and release of DA.

For NNF procedures, independent samples t-tests using slope as a dependent variable indicated that every group was significantly different from *in vitro* probe E_d . A 4 (concentration; within) $\times$ 2 (Tat vs. Veh) $\times$ 2 (Coc vs. Sal) mixed ANOVA using gain/loss scores as dependent variables indicated a main effect of Tat, $F(1, 15) = 5.97$, $p < .05$, and Concentration, $F(3, 45) = 344.03$, $p < .0001$. Furthermore, there was a Concentration X Tat Interaction, $F(3, 45) = 4.82$, $p < .5$, but no Concentration X Coc or Concentration X Tat X Coc three-way interaction, indicating that Tat treatment, but not Coc, altered the E_d when collapsing across Coc and Tat, respectively. The interaction in this ANOVA is essentially the same as a main effect of Tat when using slope

as the dependent variable, $F(1, 16) = 11.78$, $p < .01$. Figure 4 demonstrates that both Tat+Coc and Tat+Sal treated animals had significantly reduced E_d when compared to Veh+Sal ($p < .01$ and $.05$, respectively), and Tat+Coc had significantly reduced E_d when compared to Tat+Sal ($p < .01$). With respect to DA_{ext} , there was a significant elevation of basal DA levels for the Tat+Sal treated group.

3.4. Effect of Tat on cocaine-induced DA overflow dynamics 48 hrs after administration

Conventional microdialysis in Session 2 demonstrated a Tat-induced reduction in DA overflow that was augmented for animals receiving repeated Coc prior to the session. In addition, this session demonstrated an inability of Coc to induce DA overflow only in animals that were treated with Tat+Coc, suggesting a concerted effect of Tat and Coc on nerve terminal function. Within Session 2, the 8 (Time, within) X 2 (Tat vs. Veh) X 2 (Coc vs. Sal) ANOVA indicated a main effect of Tat, $F(1, 12) = 14.41$, $p < .001$, a main effect of Coc, $F(1, 12) = 5.90$, $p < .05$, and a Time X Tat Interaction, $F(7, 84) = 5.46$, $p < .0001$. The 8 X 2 ANOVA includes the 3 baseline fractions in the analysis. Thus, specific relationships between each group's DA responses to injection were analyzed with a 2 X 2 ANOVA using area under the response curve (AUC) as the dependent variable as demonstrated in Figures 5A and 5B. The 2 X 2 ANOVA upheld a main effect of Tat $F(1, 14) = 10.05$, $p < .01$, but no main effect of Coc or Tat X Coc interaction. Since the main effect of Tat collapses the Coc and Sal treated animals, each group was examined individually. Planned comparisons indicated that the Tat+Sal treated animals were reduced only relative to Veh+Sal treated animals, and that Tat+Coc treated animals were further reduced to roughly 50% of the Tat+Sal response; making their response significantly different from both Veh-treated groups.

4. Discussion

The current investigation provides *in vivo* evidence for a Tat-induced decrease in DAT function. Table 1 summarizes the effect of Tat and Coc on our measure of DAT function (E_d). Researchers have shown decreased (Wang et al., 2004; Chang et al., 2008) and increased (Gelman et al., 2006) DAT binding in HIV-infected patients. However, our data is novel in that it suggests that the altered DAT function occurs early after exposure to Tat, and prior to a significant loss of nerve-terminal function as measured by Coc's ability to incite dopamine overflow. This result in particular opens the possibility for Tat-induced alteration in DA kinetics to interact with Coc or other psychostimulants to potentiate the degradation of remaining nerve terminals. A potentiation of the decline in nerve terminal function was confirmed during Session 2, where the 3 additional injections of Coc after Session 1 moderated the effect of Tat on nerve terminal dynamics.

Within 5 hrs post-infusion, Tat effectively diminished E_d by at least 33% for animals regardless of whether they had previous exposure to repeated Coc, with no difference in DA_{ext} for any group. Table 1 summarizes the effect of Tat and Coc on DA_{ext} . The lack of difference in DA_{ext} was also demonstrated using conventional microdialysis in the second half of Session 1. Thus, the lack of difference in DA_{ext} during both approaches likely reflects decreased release offsetting the decreased uptake demonstrated during the NNF approach (Thompson et al., 2000). Combined then, decreased release and uptake suggests degeneration of nerve terminals. In addition, previous experience with Coc did not interact with Tat to moderate the decline in uptake and release.

Following NNF procedures in Session 1, conventional microdialysis demonstrated a significant increase in DA overflow to Coc challenge in both Veh and Tat treated animals, with no difference in area under the curve or peak response to Coc. The only difference between the two groups appears to manifest in a slight time delay in reaching peak response, such that it takes longer to reach peak in Veh treated animals. This slight discrepancy between groups may

be an indication of the initial stages of a Tat-induced nerve terminal reduction in release capacity.

Overall, Session 1 indicates partial depletion of nerve terminal function, in addition to an ability of remaining terminals to facilitate DA overflow following Coc administration. The ability of remaining nerve terminal to facilitate overflow has significant implications for HIV-infected Coc users. Indeed, pharmacological agents which increase extracellular DA (via D2 auto-receptor inhibition) were initially considered as CNS directed therapy for HIV-infected patients, especially given the resemblance of HIV neurological decline to that of Parkinson's disease. However, these agents were shown to acutely increase neuropsychological impairment (Hollander et al., 1985; Edelstein and Knight, 1987; Hriso et al., 1991; Factor et al., 1994). Thus, Coc's ability to facilitate DA overflow in this vulnerable system may be mechanistically linked to Coc's potentiation of Tat toxicity demonstrated here and elsewhere. Indeed, Session 2 confirmed that Coc moderated the effect of Tat by potentiating the decrease in nerve terminal function.

Specifically, NNF procedure at 48 hrs replicated the 5 h result, with the addition of two observations: 1) an exacerbated attenuation of E_d for the Tat+Coc group relative to all groups, and 2.) an increased DA_{ext} for the Tat+Sal treated group, but not for the Tat+Coc treated group (Table 1). Thus, Coc treatment appears to moderate the effect of Tat on DAT function and DA_{ext} . DA levels are the result of a balance between release and uptake kinetics (Parsons et al., 1991; Thompson et al., 2000). Thus, increased DA_{ext} , when present with decreased E_d , corresponds to either no change, or an increase in release capacity to compensate for lesioning. For example, others have noted with 6-hydroxydopamine lesions of up to 80% DA depletion, that remaining nerve terminals will increase DA turnover rates and fractional release, which in turn can be detected as a trend toward increasing DA_{ext} using NNF microdialysis (Parsons et al., 1991). In addition, decreasing DA levels typically do not manifest until 95% reduction in striatal DA (Robinson et al., 1990; Parsons et al., 1991). Thus, increased DA_{ext} in Tat + Sal animals suggests the presence of functioning nerve terminals, while decreased E_d with no change in DA_{ext} in Tat+Coc treated animals suggests perseverative degeneration of nerve terminal function.

Additional evidence for Coc's potentiation of Tat-induced nerve terminal dysfunction was demonstrated using conventional microdialysis in Session 2, whereby Coc-induced DA overflow in Tat+Coc treated animals was significantly less than every other treatment group. In fact, DA overflow in this group did not significantly elevate from baseline at any time-point, suggesting little to no response from nerve terminals in the immediate sampling area. DA response in Tat+Sal treated animals was significantly less than Veh treated animals (c.f., Ferris et al., 2009), yet contrary to Tat+Coc group, DA overflow was significantly increased throughout the sample time.

Together, results from sessions 1 and 2 have significant implications for the HIV-infected Coc dependent population. First, previous experience with Coc, as well as ensuing neuroplasticity, does not necessarily interact with the Tat protein to manifest in more severe neurodegeneration of nerve terminals. This finding is promising in the event that Coc use would cease following infection with HIV, or prior to HIV breaching the blood brain barrier (BBB). Unfortunately, without HAART therapy, breaching of the BBB occurs early in infection (An et al., 1999), and epidemiological evidence suggests that individuals who abuse Coc are significantly less likely to adhere to HAART regimens (Malone and Osborne, 2000; Hinkin et al., 2007).

Thus, a second implication is present in the likely event that Coc use continues following contraction of the virus. Namely, that a moderate amount of Coc intake after Tat administration, as modeled in the present experiment, was sufficient for potentiation of Tat-induced loss of

nerve terminal function. A possible mechanistic theory that underlies the Coc induced potentiation was recently presented (Ferris et al., 2008). Briefly, the proposed theory states that HIV-proteins may reduce DAT function and uptake kinetics, leading to an increase in extracellular DA levels; as demonstrated in the current study. This would be especially apparent following administration of Coc, which as the current study confirmed, promotes the further increase in DA levels from remaining nerve terminals. Failure of uptake to terminate the actions of DA_{ext} may then lead to D1 receptor mediated apoptotic cascades (Aksenov et al., 2006), and breakdown of DA through metabolism and/or auto-oxidative processes (Chen et al., 2008). These oxidative processes increase the amount of reactive quinones to an environment already beseeched by reactive species, leading to an extracellular environment poorly suited for nerve terminal viability (Chen et al., 2008; see Figure 3 from Ferris et al., 2008 for schematic of model). Testing the entire theory in a single design is not possible; however, much of the individual aspects have been supported in research as referenced. The current study tested an essential aspect of this theory not yet investigated. Namely, the present study confirmed 1) that Tat can degrade nerve terminal uptake and release kinetics in the striatum of an awake, freely-moving animal, 2) that despite early degradation, Coc can still induce release from remaining nerve terminals, and 3) that over time, Tat + Coc can interact to reduce both release and uptake to a larger extent than Tat or Coc alone. The first and second points explicitly support aspects of the proposed model, while the third point confirms the concerted effect of Tat and Coc on nerve terminal degradation.

Nath et al. (2001) theorized that Coc and other drugs of abuse may not increase the severity of HIV-associated neurological impairments, but may increase the rate at which impairments manifest. Thus, drugs of abuse may act as catalyst for the progression of HIV neurological decline. It is possible that with the current design, we captured the momentary status of nerve terminals as they continually decline. Thus, in animals treated with Tat + Sal, nerve terminal function could perseveratively decline and ultimately reach a floor effect equal in severity to animals treated with Tat + Coc. This is especially apparent given increased DA_{ext} in Tat treated animals from Session 2. The idea that aberrant uptake of DA and extended DA_{ext} could potentiate Tat-induced nerve terminal function is congruent with the assertion by Nath et al. (2001). Indeed, agents which increase DA levels (via D2 auto-receptor inhibition) have been shown to acutely potentiate HIV-induced neurological decline, despite early hope for many of these agents as a therapy (Hollander et al., 1985; Edelstein and Knight, 1987; Hriso et al., 1991; Factor et al., 1994).

Indeed, the development of CNS directed therapies has been impeded by the lack of data on a clear mechanism of HIV (-protein) induced neurotoxicity. The current investigation suggests the protection of nerve terminals (Bellizzi et al., 2006), and especially preservation of DAT uptake kinetics, may be a promising therapeutic avenue for HIV-infected individuals.

List of Abbreviations

aCSF	Artificial cerebrospinal fluid
ANOVA	Analysis of variance
BBB	Blood brain barrier
Coc	Cocaine

DA	Dopamine
DA_{ext}	Extracellular dopamine concentration
DAT	Dopamine transporter
E_d	Extraction Efficiency
HAART	Highly active antiretroviral therapy
HAND	HIV-Associated Neurological Decline
HIV	Human Immunodeficiency Virus
NNF	No Net Flux microdialysis
ROS	Reactive oxygen species
Sal	Saline
Tat	Transcriptional transactivator protein
Veh	Vehicle

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Figure 1. Schematic representation of probe placements as measured by post-mortem histochemical analysis. Anterior/posterior coordinates (in mm, relative to Bregma) located between +1.0 to +1.3. No animals had to be removed from the study due to a misplaced probe.

Figure 2.

NNF first-order regression from Session 1, collected the day of Tat/Veh-infusion (within 5 h for NNF). NNF demonstrates significantly reduced DAT function as measured by E_d (slope of regression) in Tat treated animals regardless of pretreatment with Coc. The E_d for both Veh treated groups was not significantly different from probe E_d , with no difference in extracellular DA levels between all groups.

Figure 3.

DA overflow data collected immediately following NNF procedures in Session 1; represented as percent of baseline across time (A) and magnitude of post-injection area under the curve (B; bar graphs). Note: Significant effects noted in graph B; * $p < .05$, ** $p < .01$ for between subjects tests of Coc vs. Sal and Veh+Coc vs. all other groups; ## $p < .01$ for between subjects tests of Tat+Coc vs. all other groups.

Figure 4.

NNF first-order regression from Session 2, collected at 48 h post-Tat or Vehinfusion. NNF demonstrates significantly reduced DAT function as measured by E_d (slope of regression) in Tat treated groups and increased extracellular content in the Tat+Sal group. Reduction in E_d is further potentiated by previous experience with Coc as indicated by the significantly reduced E_d for Tat+Coc relative to all other groups. The E_d for both all groups were significantly different from probe E_d .

Figure 5.

DA overflow data collected immediately following NNF procedures in Session 2; represented as percent of baseline across time (A) and magnitude of post-injection area under the curve (B; bar graphs). All groups were subjected to cocaine challenge, demonstrating that Tat-treatment significantly attenuates DA overflow in animals exposed to saline injection, and further reduces overflow in animals subjected to repeated Coc injections. Note: Significant effects noted in graph B; * $p < .05$, ** $p < .01$ for between subjects tests of Coc vs. Sal and Veh+Coc vs. all other groups; ## $p < .01$ for between subjects tests of Tat+Coc vs. all other groups.

Table 1
DA_{ext} and E_d across all 4 treatments 5h and 48 h following Tat-infusion.

		Vehicle		Tat ₁₋₈₆	
		Saline	Cocaine	Saline	Cocaine
DA _{ext}	5 h	1.78 ± 0.21	1.63 ± 0.36	2.10 ± 0.67	2.13 ± 0.43
	48 h	1.35 ± 0.57	2.53 ± 0.46	3.53 ± 0.93 [*]	1.54 ± 0.73 [°]
E _d	5 h	0.89 ± 0.04	0.83 ± 0.05	0.61 ± 0.06 [*]	0.61 ± 0.05 [*]
	48 h	0.84 ± 0.06	0.79 ± 0.06	0.68 ± 0.03 [*]	0.57 ± 0.02 ^{°*}

^{*} **Note:** Significantly different from Vehicle-treated, same drug control;
[°] Significantly different from Saline-treated, same treatment (Tat) control