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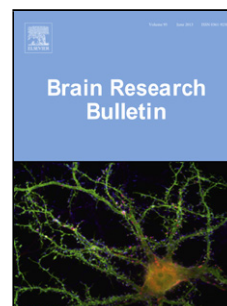
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**Effects of HIV-1 TAT protein and methamphetamine exposure on visual
discrimination and executive function in mice.**

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Running Title: TAT, METH and learning/executive function

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Highlights

- TAT expression and prior METH exposure improved visual discrimination learning.
- TAT and METH exposure impaired cognitive flexibility during reversal learning.
- TAT expression improved early, but impaired late phase reversal learning.
- Dopamine transporter levels in the caudate putamen were increased by TAT expression.

ABSTRACT

Mild neurocognitive impairments are common in people with human immunodeficiency virus (HIV) infection. HIV-encoded proteins, such as trans-activator of transcription (TAT), contribute to neuropathology and cognitive function in medicated subjects. The combination of TAT and comorbid methamphetamine use may further impair neurocognitive function in HIV-positive individuals by affecting dopaminergic systems in the brain. The current study examined the effects of TAT protein expression and methamphetamine exposure on cognitive function and dopamine systems in mice. Transgenic mice with inducible brain expression of the TAT protein were exposed to a binge methamphetamine regimen. TAT expression was induced via a doxycycline-containing diet during the final stage of the regimen and maintained throughout cognitive testing. Learning and executive function were assessed using an operant visual discrimination protocol, with a strategy switch and reversal. TAT expression and methamphetamine exposure improved visual discrimination learning. Combined TAT expression and methamphetamine exposure increased perseverative errors during reversal learning. TAT expression altered reversal learning by improving early stage, but impairing late stage, learning. TAT expression was also associated with an increase in dopamine transporter expression in the caudate putamen. These results highlight that TAT expression and methamphetamine exposure likely affect a range of selective cognitive processes, with some potentially improving function under certain conditions.

Keywords: dopamine transporter; methamphetamine binge; reversal learning; perseveration.

Abbreviations: human immunodeficiency virus (HIV), trans-activator of transcription (TAT), HIV-associated neurocognitive disorders (HAND), dopamine transporter (DAT), continuous reinforcement (CRF), phosphate-buffered saline (PBS), analysis of variance (ANOVA), methamphetamine (METH).

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1. Introduction

Mild neurocognitive impairments are common in people with human immunodeficiency virus (HIV; over 35%), even those undergoing combination antiretroviral therapy [1]. HIV-associated neurocognitive disorders (HAND) include impairments in learning, executive functions and working memory [2, 3]. Underlying corticostriatal dysfunction, such as HIV-induced decreases in caudate/basal ganglia volume [4, 5] and dopamine levels [6], may be associated with poorer cognitive performance [5, 7]. HIV-encoded proteins, such as trans-activator of transcription (TAT) and glycoprotein (gp) 120, contribute to neuronal dysfunction in the basal ganglia [8] and may explain the presence of cognitive impairments in subjects with low viral loads. For example, expression of gp120 in mice recapitulates learning deficits observed in HIV-positive humans [9].

Transgenic mice with inducible brain expression of the TAT protein [10] represent a powerful animal model to investigate the specific effects of TAT protein expression on cognitive function. Induction of the TAT protein results in neuropathology similar to that observed in HIV-infected humans [10] and leads to dysfunction in corticostriatal dopaminergic neurotransmission [11-17]. TAT exposure also induces a complex combination of cognitive dysfunction in rodents. For example, TAT impairs spatial learning [18, 19] but improves spatial reversal learning [12]. TAT expression also affects aspects of sensory motor gating [20]. However, TAT expression does not recapitulate the cognitive consequences of HIV disease entirely. For example, TAT expression does not impair delay-dependent working memory [13], even though working memory deficits are common in HIV [2, 3]. This combined evidence suggests that subcortical dopamine systems are particularly sensitive to neuropathology induced by HIV-associated proteins and this is associated with impairment of specific cognitive domains.

Corticostriatal systems are also highly susceptible to methamphetamine-induced neurotoxicity [21, 22], which is a common comorbidity in HIV-infected subjects [23]. Comorbid methamphetamine use has been associated with increased neurocognitive impairments in HIV-infected individuals [24, 25]. Furthermore, individuals who are both methamphetamine-dependent

and HIV-infected show exacerbated neuronal injury [26] and cortical interneuron loss [27, 28]. The susceptibility of corticostriatal dopaminergic systems to methamphetamine and the TAT protein, suggest their combined exposure may lead to further dysfunction. We have previously shown that TAT expression increases the sensitivity to methamphetamine reward [13] and enhances methamphetamine-induced sensitization [14]. However, their combined influence on cognitive function is understood less.

The aim of the present study was to determine the combined effects of TAT protein expression and methamphetamine exposure on cognitive function in mice. To effectively model methamphetamine use and TAT expression in human subjects, we combined the temporal control of TAT expression in inducible transgenic mice [10] with a binge regimen of methamphetamine exposure that closely resembles use in human subjects [22]. Subsequent to methamphetamine exposure, learning and executive function were assessed using an operant visual discrimination protocol. This protocol featured the acquisition of a visual discrimination rule as a measure of learning, followed by strategy switch and reversal stages to assess executive function. To assess the effects of TAT and methamphetamine on brain dopaminergic systems, dopamine transporter (DAT) levels were determined in mesolimbic and striatonigral brain areas.

2. Methods

2.1 Animals

A total of 48 male mice (9-14 months old at the beginning of the experiment) were provided by the Neuroscience and Animal Models Core of the Translational Methamphetamine AIDS Research Center (TMARC) and tested. Of these, 22 mice contained the GFAP promotor-controlled Tet-binding protein (TAT-) and 26 contained both the GFAP promotor-controlled Tet-binding protein and the TRE promotor-TAT protein transgene (TAT+). There were no significant differences in ages between the test groups (TAT-/saline, 12.6 ± 0.3 [SEM]; TAT+/saline, 12.4 ± 0.4 ; TAT-

/methamphetamine, 12.6 ± 0.5 ; TAT+/methamphetamine, 12.9 ± 0.4). Inducible TAT transgenic mouse colonies with a C57BL/6J background were obtained by generation of two separate transgenic lines Teton-GFAP mice and TRE-Tat86 mice, and then cross-breeding of these two transgenic mouse lines, as previously described [10]. The mice were housed 1-4 mice per cage in a humidity- and temperature-controlled animal facility on a 12 h/12 h reverse light/dark cycle (lights off at 7:00 AM). Mice had *ad libitum* access to food and water during exposure to methamphetamine binge (1-3 weeks). As doxycycline has been shown to induce behavioral changes when administered to control mice [13], all mice were given a doxycycline-containing diet (6 g/kg diet #F4096, Bioserv Flemington, NJ). Mice were provided with the diet on day 21 of methamphetamine exposure (i.e., two days prior to cycle 4 of administration) and continuing throughout the experiment (see **Table 1**). Inducing TAT expression via chronic exposure to a doxycycline-containing diet has been demonstrated to produce functional changes to synaptic physiology and alterations in behavior [29]. During behavioral training and testing, mice were food deprived to 85% free-feeding weight, but had *ad libitum* access to water. Behavioral testing was conducted 7 days/week during the dark phase of the light/dark cycle from 2 pm to 7 pm with mice from all groups being tested concurrently at any given time throughout the testing period. All of the experiments were conducted in accordance with the guidelines of the American Association for the Accreditation of Laboratory Animal Care and National Research Council's Guide for the Care and Use of Laboratory Animals and approved by the University of California San Diego Institutional Animal Care and Use Committee.

2.2 Experimental Design

Mice underwent the binge methamphetamine regimen and were placed on a doxycycline containing diet two days prior to the final cycle of the regimen. Mice remained on this diet for all subsequent behavioral testing. Visual discrimination training began two weeks after completion of

the binge regimen. The advanced age of the mice tested led to approximately 30% of the mice failing to reach criteria independent of TAT expression and methamphetamine exposure. This may also be a consequence of doxycycline administration. We have previously observed behavioral changes as a direct result of doxycycline administration in TAT- mice [13], which is why doxycycline treated TAT- mice were used as controls in the present study. An equivalent control group fed a diet without doxycycline would be required to establish age-dependent versus doxycycline-dependent effects on task completion. Thus, final group numbers for behavioral data were: TAT-/saline = 7, TAT+/saline = 9, TAT-/methamphetamine = 9, and TAT+/methamphetamine = 7. After completion of behavioral testing, mice underwent anaesthetized magnetic resonance imaging (these data are not presented in this manuscript) and then brain samples from all mice (15-20 months old) were collected for immunohistochemistry analyses (TAT-/saline = 12, TAT+/saline = 13, TAT-/methamphetamine = 8, and TAT+/methamphetamine = 10).

2.3 Methamphetamine regimen

The methamphetamine binge regimen was previously developed in our laboratory to mimic human binge use parameters [22]. The binge consisted of subcutaneous injections of saline or methamphetamine (methamphetamine hydrochloride; Sigma, St. Louis, MO, USA; reported as base concentration) for four ‘cycles’ of 4 days (four injections/day) with 3 days between cycles (**Table 1**). The first cycle featured one week of dose-escalation followed by three repeated identical cycles of methamphetamine exposure. These 2-4 cycles consisted of a two-day dose-escalation (3-5 mg/kg) followed by two days of high dosing (6 mg/kg). Saline and methamphetamine were administered at a volume of 5 ml/kg.

<<<<Insert Table 1>>>>

Table 1. Methamphetamine binge regimen

Cycle	1				2				3				4			
	1	2	3	4	9	10	11	12	16	17	18	19	23	24	25	26
12:00	1	3	5	6	3	5	6	6	3	5	6	6	3	5	6	6
14:00	1	3	5	6	3	5	6	6	3	5	6	6	3	5	6	6
16:00	2	4	5	6	4	5	6	6	4	5	6	6	4	5	6	6
18:00	2	4	5	6	4	5	6	6	4	5	6	6	4	5	6	6

Methamphetamine doses are reported as base concentration (mg/kg).

<<<<End Table 1>>>>

2.4 Visual discrimination task

2.4.1 Discrimination training

Visual discrimination training and testing were conducted in twelve Plexiglas operant chambers (model ENV-307A, Med Associates Inc., St. Albans, VT, USA), each enclosed in a sound-attenuating cubicle. The task consisted of 2 continuous reinforcement (CRF) training stages followed by the visual discrimination test phase. During the first CRF stage, a single lever was presented (left or right, chosen at random). A single lever press resulted in the delivery of a single food reward (20 mg Sucrose pellet, TestDiet, IN, USA). The criterion to progress to the next stage was >30 lever presses over three days. The second CRF stage was identical, except the lever retracted after each press. Following a nose poke in the reward receptacle (15 s hold), a 5 s delay was initiated before the lever was extended for the next trial. The criterion to progress to the next stage was >40 lever presses with <30% omissions in two of three days. For the visual discrimination task, both levers were extended and one of the stimulus lights above the lever was illuminated. The mouse was required to press the lever with the associated stimulus light illuminated. An incorrect response or failure to respond to either the sample or choice levers during the 20 s limited hold (i.e., an omission) resulted in a time out period (house lights on) of 5 s. Correct responses were calculated as a percentage of the total trials (not including omissions). The criterion to complete visual discrimination and progress to the strategy switch phase was >70% correct trials and <30% omissions over three days. Mice were given 60 trials per test session.

2.4.2 Strategy switch

Once criteria were met for visual discrimination stage, mice continued onto strategy switch testing. All the parameters for testing remained the same as for the visual discrimination test but the reward contingencies were changed. Rather than the stimulus light predicting the rewarded lever, the spatial location of the lever (left or right) was the relevant reward contingency. The criterion to complete testing for strategy shift and progress to reversal learning testing was >70% correct trials and <30% omissions over three days.

2.4.3 Spatial reversal learning

Once criteria were met for the strategy switch stage, mice continued onto reversal learning testing. All the parameters remained the same as for the strategy switch test but the reward contingencies were changed. For mice where the left lever had previously predicted the reward, the right lever was now the correct contingency, and *vice versa* where the left lever had previously predicted the reward. The criterion for completion of reversal learning testing was >70% correct trials and <30% omissions over three days.

2.4.4 Behavioral measures

Behavioral measures included the amount of trials (total, correct and error trials) to reach criterion. Latency measures including the amount of time (ms) the animals took to respond and to collect the food reward were also recorded. The specific strategies used were also recorded including win-stay and lose-shift. That is, following a rewarded trial, a win-stay event was recorded if a subject made a response at the same lever. Conversely, following an incorrect response, a lose-shift event was recorded if a subject made a response at the opposite lever.

Additional measures were obtained for reversal learning. Specifically, the number of perseverative errors, defined as the total number of responses on the previously rewarded lever until a correct response was recorded. Further, the number of trials was broken down into early and late

learning phases [30]. To define early vs late phase learning, each session was split into blocks of 20 trials. Once mice reached >50% correct responses in a given block, this and all subsequent blocks were considered late phase learning.

2.5 Dopamine transporter quantification

Briefly, as previously described [31], mouse hemibrain tissue sections were deparaffinized using xylene followed by rehydration in serial ethanol and water solutions. Next, tissue sections were treated for 30 min with 3% hydrogen peroxide/phosphate-buffered saline (PBS) and then incubated for 30 min with 2.5% normal serum, corresponding to the host species for the secondary antibody. Tissue sections were then incubated with anti-DAT (Santa Cruz Biotechnology, Dallas, TX; Cat# sc-32258; 1:100 in PBS) for 2 h at room temperature in a hydration box. Subsequently, tissue sections were washed with 0.1% Tween 20/PBS, before 30 min incubation with horse anti-rabbit IgG peroxidase-polymer secondary antibody (ImmPRESS, Vector Laboratories, Burlingame, CA, USA). After the tissues were washed with 0.1% Tween 20/PBS, the signals were developed with diaminobenzidine (ImmPACT DAB peroxidase substrate, Vector Laboratories) for 5 min. The immunostained sections were then dehydrated via serial ethanol and water solutions, de-waxed with xylene, and mounted using Cytoseal 60 (ThermoScientific). For the negative control, the primary antibody was omitted.

Subsequently, immunostained sections were scanned using a microscope slide scanner (Aperio ScanScope GL, Leica Biosystems, Buffalo Grove, IL, USA) equipped with a 20× objective lens (yielding the resolution of 0.5 μm per pixel). Assessment of levels of DAT immunoreactivity was performed using the Aperio ImageScope software. For each case a total of three sections (5 images per section) were analyzed to estimate the average optical density of immunolabelled cells per unit area (mm^2). Corrected optical density was calculated by subtracting the background optical density

of the negative control (obtained from tissue sections immunostained in the absence of primary antibody) from the optical density of the immunostained sections.

2.6 Statistical analyses

All analyses were performed with IBM SPSS Statistics 20 (Armonk, NY, USA). Data were analyzed using analysis of variance (ANOVA), with *TAT* and *Methamphetamine* as the between-subject factors. Repeated-measures ANOVAs were used when within-subject factors were present. Data not meeting the assumption of homogeneity of variance were analyzed using Greenhouse-Geiser adjusted degrees of freedom. When appropriate, post hoc comparisons were performed using Least Significant Difference (LSD) analyses. Perseverative errors were analyzed non-parametrically using a Kruskal-Wallis ANOVA. Results are expressed as mean \pm SEM. Differences were considered statistically significant at $p < 0.05$.

3. Results

3.1 Visual discrimination

For the trials required to reach criterion, there were significant interactions of *Methamphetamine* x *TAT* for total trials (**Fig 1A**; $F_{1,28}=4.6$, $p < 0.05$) and correct trials (**Fig 1B**; $F_{1,28}=6.1$, $p < 0.05$). There was also a trend towards an interaction of *Methamphetamine* x *TAT* for error trials (**Fig 1C**; $F_{1,28}=3.1$, $p < 0.1$ NS). *TAT* expression and methamphetamine exposure alone, but not in combination, improved visual discrimination learning as demonstrated by a significant decrease in the total and correct trials to reach criterion. There was also a trend ($p < 0.1$) for combined *TAT* expression and methamphetamine exposure to decrease the number of correct trials to reach criterion (**Fig 1B**).

There were no significant effects or interactions of *TAT* or *Methamphetamine* on the response latency (*Fig S1A-C*) or win-stay/lose-shift strategies used during visual discrimination learning (*Fig S2A*).

3.2 Strategy Shift

Neither *TAT* expression nor methamphetamine exposure significantly altered the total trials (*Fig S3A*), correct trials (*Fig S3B*) and error trials (*Fig S3C*) to reach criterion. There were also no significant effects or interactions of *TAT* or *Methamphetamine* on the response latency (*Fig S1D-F*) or win-stay/lose-shift strategies used during the strategy switch (*Fig S2B*).

3.3 Spatial reversal learning

Neither *TAT* expression nor methamphetamine exposure significantly altered the total trials (*Fig S3D*), correct trials (*Fig S3E*) and error trials (*Fig S3F*) to reach criterion. There were no significant main effects or interactions of *TAT* or *Methamphetamine* on the response latency (*Fig S1G-I*) or win-stay/lose-shift strategies used during the strategy switch (*Fig S2C*). However, there was a significant difference between the groups on the number of perseverations (**Fig 2**; $H_{(3)}=8.5$, $p<0.05$). The control group showed the lowest level of perseverations, followed by the methamphetamine exposed group and the *TAT*-expressing group, with the greatest level of perseverative errors in the methamphetamine exposed, *TAT*-expressing group. Post hoc tests confirmed that the methamphetamine exposed, *TAT*-expressing group made significantly more perseverative errors than control mice ($p < 0.05$).

Reversal learning features two distinct phases of learning [30]: an early phase (<50% correct responses) whereby the animal must learn to disassociate the prior reward contingencies, and a late

phase (>50% correct responses) whereby the animal must learn the new associations. The different phases can be independently altered after a variety of manipulations [30]. Compared with the late phase, the early phase featured less total (**Fig 3A**; $F_{1,28}=71.8$, $p<0.001$) and correct trials (**Fig 3B**; $F_{1,28}=383.6$, $p<0.001$) but more errors trials (**Fig 3C**; $F_{1,28}=17.4$, $p<0.001$). Moreover, there was a significant interaction of *Phase* x *Methamphetamine* x *TAT* for error trials ($F_{1,28}=4.6$, $p<0.05$) and a similar trend for total trials ($F_{1,28}=3.4$, $p<0.1$ NS). TAT expression tended to decrease early phase errors and increase late phase errors (**Fig 3C**) compared with controls, methamphetamine exposure or combined TAT expression and methamphetamine exposure. A similar pattern was observed for total trials during early and late phase learning (**Fig 3A**). There were no significant effects or interactions of *TAT* or *Methamphetamine* during early or late phase learning for response latency (*Fig S4A,B*) or win-stay/lose-shift strategies (*Fig S4C,D*).

3.4 Dopamine transporter expression

In the caudate putamen, there was a significant interaction of *Methamphetamine* x *TAT* for dopamine transporter expression ($F_{1,35}=5.2$, $p<0.05$). TAT expression significantly elevated DAT levels compared with controls, methamphetamine exposure and combined TAT expression and methamphetamine exposure (**Fig 4A**). There were no significant effects or interactions of *TAT* or *Methamphetamine* on dopamine transporter expression in the nucleus accumbens (**Fig 4B**), substantia nigra (**Fig 4C**) or ventral tegmental area (**Fig 4D**).

4. Discussion

The results show a complex pattern of outcomes after TAT expression and/or methamphetamine exposure. That is, TAT expression and methamphetamine exposure both independently improved visual discrimination learning. TAT expression improved early phase reversal learning, at the

expense of late phase reversal learning. TAT expression was also associated with increased dopamine transporter levels in the caudate putamen. Surprisingly, the combination of TAT expression and methamphetamine exposure did not induce significant learning deficits, but did increase perseverations at the initiation of reversal learning suggesting slight impairments in executive function. Thus, TAT expression showed subtle effects on learning that may be due to alterations in dopaminergic function, but these effects are not summative or synergistic with those of methamphetamine.

Contrary to our expectations, TAT expression and methamphetamine exposure improved learning in a visual discrimination task, decreasing the total and correct trials to reach criterion. HIV-associated proteins such as gp120 [32], as well as methamphetamine exposure [33], have been shown to alter hypothalamic-pituitary-adrenal axis function, which may alter the motivation toward food rewards in operant behavioural tasks. However, this is unlikely as the improvement observed in the present study was not associated with differences in response latency or the strategies used during the task. Although a similar pattern was observed for error trials, the greater magnitude and larger effect on the correct trials required would suggest that this outcome may be attributed to an increased sensitivity to positive feedback. Rats previously exposed to methamphetamine show an increased sensitivity to, and reliance on, positive feedback during discrimination tasks [34]. There is also evidence that HIV-positive individuals direct more attention towards reward-associated information [35]. Thus, both TAT and methamphetamine exposure can increase the perceived relevance of positive feedback, which may explain the present findings. Reinforcement learning is heavily associated with dopamine function in mesolimbic projections [36]. We have shown that this regimen of methamphetamine exposure induces a persistent decrease in dopamine within the nucleus accumbens [22] and that TAT expression also tends to decrease dopamine in the nucleus accumbens [13]. Combined TAT expression and methamphetamine exposure tended to show the same pattern of improvement; however, this effect was not significant. The effects of dopamine levels on cognitive function has a well-established inverted-U-shaped relationship [37], with

optimal levels of dopamine required for peak performance (i.e, too much or too little dopamine can lead to impaired performance). Thus, it is possible that combined effects of TAT expression and methamphetamine exposure on dopamine systems are too large, and performance gains are no longer evident.

TAT expression and methamphetamine exposure induced a complex pattern of effects on reversal learning in the present study. Specifically, combined TAT expression and methamphetamine exposure increased the level of perseverative responses at the initiation of reversal learning. The orbitofrontal cortex and caudate putamen are critical for reversal learning [38-40]. The methamphetamine regimen used in the current study led to persistently increased dopamine levels in the orbitofrontal cortex and decreased dopamine levels in the caudate putamen [22]. Alterations in the neurochemistry of these key regions, combined with TAT expression, could explain the increase in perseverative errors observed after combined TAT expression and methamphetamine exposure.

In contrast, TAT expression alone improved early phase reversal learning at the expense of late phase reversal learning. Given this effect was associated with selectively increased dopamine transporter levels in the caudate putamen of this group, it is tempting to conclude the two are associated. However, it is likely more complex as differing factors appear to mediate early and late phase reversal learning. For example, selective improvements in early phase reversal learning have been observed after increasing serotonin levels in the brain [30]. The selectivity of serotonin alterations to early phase reversal learning suggests that early and late phase reversal learning may be independent processes. TAT expression has been shown to increase serotonin levels in the nucleus accumbens [13] suggesting this may be a potential mechanism for the observed early phase improvements. However, whether cortical serotonin levels are altered is not known and how influential this may be in HIV infection remains to be determined. Most evidence suggests that serotonin level/function is decreased after HIV infection. For example, polymorphisms that reduce serotonin function impair cognitive performance in HIV-positive subjects [41] and Simian

Immunodeficiency Virus infection in Macaques decreases serotonin levels [42]. This provides further evidence that the global effects of HIV disease are the result of multiple factors in combination and individual proteins/responses represent only one contributing factor in the symptom profile of HIV disease. In contrast to early phase learning, the caudate putamen is crucial for establishing and maintaining the new rule in late phase learning. For example, lesions of the medial caudate putamen impair reversal learning in rats by increasing the number of errors made when learning and maintaining the new rule [39]. Thus, alterations in dopamine signalling, due to increased dopamine transporter levels in the caudate putamen, may have selectively impaired late phase learning in TAT-expressing mice. This would be consistent with our prior work, whereby cessation of TAT protein expression was associated with recovery of dopamine indices and improvements in late phase reversal learning [12, 13].

No significant effects of TAT expression and methamphetamine exposure were observed on the strategy-switch phase of testing. The prefrontal cortex is critical for shifting from one rule or strategy to another [38-40] so this would suggest that prefrontal cortex function is not impaired under the current experimental conditions.

In conclusion, both TAT expression and methamphetamine exposure may improve associative learning under certain experimental conditions by enhancing the response to positive feedback. However, an over-emphasis on positive feedback would not be beneficial in more challenging and less predictable real-world situations i.e., gambling. Furthermore, an increased drive for positive feedback due to TAT expression may make it more difficult for HIV-infected subjects, who are dependent, to abstain from using methamphetamine. These results highlight the complexity of modelling and understanding HIV disease and methamphetamine abuse. The HIV-associated TAT protein likely affects a range of factors, with some potentially improving function under certain conditions.

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FIGURE LEGENDS

Fig 1. Visual discrimination learning.

Total (A), correct (B) and error (C) trials to reach criterion on the visual discrimination task in TAT-expressing (TAT+) and/or methamphetamine-exposed (METH+) mice. Both TAT-expressing (TAT+/METH-) and methamphetamine-exposed (TAT-/METH+) mice required less trials to reach criterion than control (TAT-/METH-) mice. This effect was most evident in the total (A; *Methamphetamine* x *TAT* [$F_{1,28}=4.6$, $p<0.05$]) and correct (B; *Methamphetamine* x *TAT* [$F_{1,28}=6.1$, $p<0.05$]) trials required. A similar but non-significant trend was also observed for the number of errors (C; *Methamphetamine* x *TAT* [$F_{1,28}=3.1$, $p<0.1$ NS]). Data are expressed as mean \pm SEM. * $p < 0.05$ compared to TAT-/METH-.

Fig 2. Perseverative errors during reversal learning

Perseverative errors at the initial reversal of reward contingencies in TAT-expressing (TAT+) and/or methamphetamine exposed (METH+) mice. TAT+/METH+ mice made significantly more perseverative errors compared with the control (TAT-/METH-) group ($H_{(3)}=8.5$, $p<0.05$). Data are expressed as mean \pm SEM. * $p < 0.05$.

Fig 3. Early (<50%) and late phase (>50%) reversal learning

Total (A), correct (B) and error (C) trials to reach criterion during the early (<50%) and late phases (>50%) of reversal learning in TAT-expressing (TAT+) and/or methamphetamine exposed (METH+) mice. A significant decrease in error trials was observed during the late phase compared with the early phase of learning in all groups except for the TAT+/METH- mice (C; *Phase* x *Methamphetamine* x *TAT* [$F_{1,28}=4.6$, $p<0.05$]). TAT+/METH- mice tended to make less early phase errors and more late phase errors compared with the other groups. Data are expressed as mean \pm

SEM. *** $p < 0.001$. # $p < 0.05$, ### $p < 0.001$ when comparing <50% with >50% learning phases within group.

Fig 4. Dopamine transporter expression

Relative dopamine transporter pixel intensity in the caudate putamen (**A**), nucleus accumbens (**B**), substantia nigra (**C**) and ventral tegmental area (**D**) in TAT-expressing (TAT+) and/or methamphetamine exposed (METH+) mice. TAT+/METH- mice had significantly greater dopamine transporter expression in the caudate putamen compared with all other groups (**A**; *Methamphetamine* x *TAT* [$F_{1,35}=5.2, p<0.05$]). Data are expressed as mean \pm SEM. * $p < 0.05$, ** $p < 0.01$.

