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Chronic methamphetamine exposure exerts few effects on the iTat mouse model of HIV, but blocks Tat expression-induced slowed reward retrieval

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Abstract

Human immunodeficiency virus (HIV) continues to infect millions worldwide, negatively impacting neurobehavioral function. Further understanding of the combined effects of HIV and methamphetamine use is crucial, as methamphetamine use is prevalent in people with HIV. The HIV-associated protein Tat may contribute to cognitive dysfunction, modeled preclinically in mice using doxycycline (DOX)-inducible Tat expression (iTat). Tat may exert its effects on cognitive function via disruption of the dopamine transporter, similar to the action of methamphetamine. Additionally, Tat and methamphetamine both decrease interneuron populations, including those expressing calbindin. It is important to understand the combined effects of Tat and methamphetamine in preclinical models of HIV infection. Here, we used iTat transgenic mice and a chronic binge regimen of methamphetamine exposure to determine their combined impact on reward learning and motivation. We also measured calbindin expression in behavior-relevant brain regions. Before induction with DOX, iTat mice exhibited no differences in behavior. Chronic

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CRediT authorship contribution statement

Jared W. Young : Conceptualization, Funding acquisition, Formal analysis, Project administration, Methodology, Resources, Supervision, Writing – original draft, Writing – review & editing. **Johnny A. Kenton :** Formal analysis, Writing – original draft. **Morgane Milienne-Petiot :** Investigation, Formal analysis. **Debbie Deben :** Investigation, Formal analysis. **Cristian Achim :** Project administration, Resources. **Mark A. Geyer :** Resources, Funding acquisition. **William Perry :** Resources, Funding acquisition. **Igor E. Grant :** Resources, Funding acquisition. **Arpi Minassian :** Conceptualization, Funding acquisition, Writing – original draft, Writing – review & editing.

Appendix A. Supporting information

Supplementary data associated with this article can be found in the online version at doi:10.1016/j.bbr.2022.114109.

Uncited references

[28,44].

methamphetamine exposure before Tat induction impaired initial reward learning but did not affect motivation. Furthermore, DOX-induced Tat expression did not alter behavior, but slowed latencies to retrieve rewards. This effect of Tat, however, was not observed in methamphetamine-treated mice, indicative of a potential protective effect. Finally, Tat expression was associated with an increase in calbindin-expressing cells in the VTA, while methamphetamine exposure did not alter calbindin numbers. These findings may indicate a protective role of methamphetamine in HIV neuropathology, which in turn may help in our understanding of why people with HIV use methamphetamine at disproportionately higher rates.

Keywords

ITAT HIV model; Methamphetamine; Mouse; Operant; Reinforcement; Motivation

1. Introduction

Since its discovery in the 1980s, nearly 76 million people globally have become infected with human immunodeficiency virus (HIV), with approximately 33 million deaths related to infection [1]. As recently as 2019, approximately 38 million people (~36 million adults) were living with HIV, although the rate of new infections has declined by 23 % in the past decade [20]. Although recent antiretroviral therapy (ART) developments have reduced mortality and improved quality-of-life, several HIV-associated effects have long-lasting impacts on the lives of people with HIV. One such effect is due to neuronal damage, which can lead to HIV-associated neurocognitive disorders (HAND) (reviewed in [8,24,62], regardless of ART. Indeed, previous research has revealed HIV-associated deficits in attention, motor function, motivation, executive control, and memory al. [37,16,47]. Since HIV does not infect neurons directly [38], however, it is imperative that efforts focus on determining the mechanism(s) by which HIV-associated neuropathology occurs.

Several HIV-associated viral proteins have been implicated in HAND, including gp120 (an envelope glycoprotein) and the transactivator of transcription (‘Tat’; a regulatory protein), both of which are associated with neurologic injury [12,29], where over-expression of either protein impairs reinforcement learning and memory [22,27]. The non-structural Tat protein encoded in the genes of HIV enhances the efficiency of viral transcription [9], and exhibits both direct and indirect pathway effects resulting in neuronal damage. The direct pathway involves release of Tat from infected cells and direct interaction with neurons, while the indirect pathway includes dysregulation of inflammatory processes and oxidative stress [43]. One direct interaction between Tat and neurons that is the focus of this study is the ability of Tat to interfere with the function of the dopamine transporter (DAT), which recycles dopamine from the synaptic cleft. Dopamine is important for food seeking [11], Pavlovian approach behavior [49], reinforcement learning [18], and motivated behavior [48]. Such dopaminergic effects implicate DAT in the dysfunction observed with HIV [15].

DAT function, however, is also influenced by use of methamphetamine (methamphetamine), such that methamphetamine abuse negatively impacts cognitive functioning. For example, chronic methamphetamine use affects domains of episodic memory, executive control,

and information processing speed in otherwise healthy people [52]. Relative to healthy populations, methamphetamine use is much higher in people with, or at risk for, HIV [10,40]. methamphetamine exacerbates the viral load of HIV [13], and HIV becomes more infectious in neural progenitor cells in the presence of methamphetamine [54]. Previous exposure to methamphetamine can alter activity levels in mice, indicating that methamphetamine can exert effects even after withdrawal [21,22].

Furthermore, methamphetamine reduces calbindin (CB)-expressing cells in rat [34], an effect that has also been observed in the post-mortem brains HIV-positive methamphetamine users [35]. Research shows that HIV patients with a history of methamphetamine abuse exhibit memory deficits correlated with damage to CB-immunoreactive interneurons in the neocortex and striatum [35]. In a rat model, Kuczenski and colleagues [34] demonstrated a significant decrease in CB neuronal immunoreactivity of the neuropil in the hippocampal CA2 and CA3 regions, while the CB-immunoreactive neuronal population was relatively preserved after methamphetamine exposure. While the behavioral effects of certain aspects of HIV (e.g., the envelope glycoprotein gp120) in conjunction with methamphetamine exposure have been researched [21], the combined effects of methamphetamine and Tat expression are less explored, particularly with regard to reward learning and motivation, and its impact on CB expression.

In this study, we explored the potential interactions between Tat expression and methamphetamine effects. We utilized a Tat transgenic mouse model (iTat) and a chronic exposure model of methamphetamine exposure to assess goal-directed behavior. Additionally, we assessed numbers of CB-expressing interneurons in brains regions related to such behavioral performance (prefrontal cortex, hippocampus, nucleus accumbens, and ventral tegmental area). We hypothesized that expression of the Tat protein combined with chronic methamphetamine exposure would exacerbate goal-directed impairments in iTat mice, associated with a loss of CB-expressing cells, particularly in the domains of reinforcement learning and motivation.

2. Methods

2.1. Animals

A total of 60 male mice (31 WT, 29 Tat) on a C57Bl/6J background were housed in a maximum of four mice per cage and maintained in a temperature-controlled vivarium (21 ± 1 °C) on a 12 h/12 h reversed light-dark cycle (lights on 19:00, lights off 7:00). Mice negative for Tat (WT) contained GFAP promoter-controlled Tet-binding protein (TGFAP+) while mice positive for Tat (iTat) contained TGFAP+ promotor *and* the TRE promotor-Tat protein transgene (TAT86+). All mice were supplied by Dr. Marcus Kaul and kept in quarantine for 8 weeks prior to the beginning of training. All mice were given ad libitum access to water and were food restricted to approximately 85 % of their free-feeding body weight, starting at least one week before training began. Testing occurred in the dark phase (13:00–18:00). All procedures were approved by the UCSD Institutional Animal Care and Use Committee. The UCSD animal facility meets all federal and state requirements for animal care.

2.2. Drug regimens

The methamphetamine regimen was administered as described previously [25,26]. Briefly, mice were administered injections (s.c.) of saline (0.9 %) or methamphetamine hydrochloride (5 mL/kg; Sigma, St. Louis, MO, USA) for a 25-day regimen (4-day block of injections separated by 3 days of washout; four injections/day at 12:00, 14:00, 16:00, and 18:00) (Fig. 1). Drug concentrations were gradually increased over time, starting with 1 mg/kg on day one up to 6 mg/kg on day 25. This binge regimen was selected to fit models of total use per day/month in humans.

Toward the end of the methamphetamine regimen (day 23), all mice were treated with a doxycycline (DOX; doxycycline hyclate; Sigma, St. Louis, MO, USA) regimen of 100 mg/kg (i.p.) daily for 7 days, paired with the first injection of the methamphetamine regimen (i.e., at 12:00), consistent with previous studies (Fig. 1) [26]. This DOX regimen was to induce Tat expression in the iTat mice, while having no effect on expression in WT mice, importantly occurring after heavy methamphetamine use. During the 25-day period, mice were weighed twice per week to determine the injection volume for the following days.

2.3. Operant tests

2.3.1. Training—The apparatus used for operant training consisted of a chamber with one curved rear wall, containing five holes. All chambers were enclosed in ventilated, sound-attenuating cabinets (Med Associates Inc., St. Albans, VT, USA and Lafayette Instrument Company, Lafayette, IN, USA). The holes in each chamber contained LEDs and infrared beams to detect mouse nose-poking into the hole. Only holes 1 and 2 were used for operant training. When a nose-poke was detected in the illuminated hole, strawberry milkshake (Nesquik (Vevey, Switzerland) plus non-fat milk, 30 μL) was delivered into the reward magazine located on the opposite wall. Mice were first trained to retrieve liquid reward from an illuminated hole delivered into the reward magazine every 15 s. After receiving 50 rewards in a 30 min session over 2 consecutive days, mice were then trained to nose poke in individually illuminated holes to receive reward (FR1). Mice progressed to the next stage after obtaining 70 rewards in 30 min over 2 consecutive days. Additionally, all mice performed “maintenance” sessions of FR1 to ensure continued responding (4 during methamphetamine treatment and 4 during washout periods). All training and testing programs were controlled by a SmartCtrl 8-in/16-out Package with additional interfacing by MED-PC for Windows (Med Associates Inc., St. Albans, VT, USA) using custom programming (in-house). All operant testing occurred at 3 specific time points relative to drug delivery: pre-methamphetamine (baseline testing), during-methamphetamine (at the end of methamphetamine regimen), and post-methamphetamine (6 days after final methamphetamine administration).

2.3.2. Probabilistic Learning Task (PLT)—After passing FR1 training, mice were tested in the PLT, wherein mice chose between two illuminated holes (target vs. non-target; reward: punishment = 90 %: 10 %). Similar to FR1 training, correct responses resulted delivery of strawberry milkshake (30 μL) in the reward magazine. Punishment involved an absence of reward and a time-out period with illumination of the house light for 4 s. Target holes were those in which each mouse performed the fewest nose-pokes during the FR1

training. Testing involved illumination of only holes 1 and 2 and lasted until mice performed 60 trials. The primary measures for PLT were accuracy, mean reward latency, and mean target latency.

2.3.3. Progressive ratio breakpoint task (PRBT)—After completing the PLT, mice were moved to the progressive ratio breakpoint task (PRBT). In this task, mice were required to nose poke for reward into hole 3 (located in the center, across from the reward magazine) when illuminated. The number of nose-pokes required to complete a trial increased after three reward deliveries, such that mice were to nose poke 4 times, then 7, then 11, and so on [59]. The primary outcome measures of the PRBT was the "breakpoint," defined as the last ratio to be completed before the end of the session. Secondary measures were the mean reward latency and the mean reaction time.

2.4. Histology

Mouse brain hemispheres were fixed for 24 h in 4 % paraformaldehyde and then embedded in paraffin. 5 μm thick sagittal sections were processed for immunostaining using a rabbit polyclonal anti-Calbindin antibody (MilliporeSigma, Burlington, MA, USA) and the signal was detected with the ImmPRESS peroxidase-micropolymer anti rabbit secondary antibody and ImmPACT DAB substrate (Vector® Laboratories, Burlingame, CA, USA).

Stained mouse brain sections were scanned with the Aperio ScanScope glass slide scanner using the Aperio ImageScope software. Parameters regarding the specific brain regions and microscope-specific orientations were used consistently through all analyses. Intensity and Area values were calculated for each sample using Microsoft Excel.

2.5. Statistical analyses

All statistical analyses were performed using IBM SPSS Statistics 27 (Armonk, NY, USA). Operant maintenance data was assessed using a Student's t-test. All other behavioral and molecular data were analyzed using mixed analysis of variance (ANOVA), with drug and genotype as between-subjects factors, and where applicable, trialblock for within-session learning as a within-subjects factor. Tukeys post hoc analyses were conducted on significant main effects and their interactions. Results are expressed as mean ± standard error of the mean (SEM). Differences were considered statistically significant at $p < 0.05$.

3. Results

3.1. Baseline performance

3.1.1. Probabilistic learning task—During baseline training in the PLT, both WT and iTat mice exhibited similar performance in accuracy ($t(50) = 0.624$, $p = 0.535$; Fig. 2A). Additionally, mice were no different in secondary measures of time to make a correct response ($t(50) = -1.003$, $p = 0.320$; Fig. 2B) or time to retrieve rewards ($t(50) = 0.831$, $p = 0.410$; Fig. 2C).

3.1.2. Progressive ratio breakpoint task—During baseline training in the PRBT, both WT and iTat mice exhibited similar breakpoints ($t(50) = 0.270$, $p = 0.789$; Fig. 2D).

WT and iTat mice were also no different in response times ($t(50) = 0.146$, $p = 0.885$; Fig. 2E) or latencies to acquire rewards ($t(50) = 0.390$, $p = 0.659$; Fig. 2F).

3.2. Maintenance testing

FR1 maintenance sessions occurred both with methamphetamine off- (methamphetamine-Off) and methamphetamine on-board (methamphetamine-On). During methamphetamine-Off maintenance sessions, all mice performed above criterion (75 responses) and groups were generally similar across sessions (Saline vs. methamphetamine; Session 1: $t(43) = 0.607$; $p = 0.547$; Session 3: $t(40) = -0.800$; $p = 0.429$; Session 4: $t(34) = -1.702$; $p = 0.098$), with the exception of Session 2, where the methamphetamine group displayed worse performance (Session 2: $t(34) = 2.772$; $p = 0.009$; Fig. 3A). During methamphetamine-On sessions, saline-treated mice performed above criterion across all four sessions, while methamphetamine mice only performed above criterion on the fourth session. Additionally, methamphetamine-treated mice demonstrated significantly worse performance compared to saline-treated mice on sessions 1–3 (Session 1: $t(40) = 11.955$; $p < 0.005$; Session 2: $t(31) = 15.318$; $p < 0.005$; Session 3: $t(43) = 4.388$; $p < 0.005$), but not on session four (Session 4: $t(34) = 1.895$; $p = 0.067$; Fig. 3B). Due to the temporal effect of methamphetamine on-board on operant responding, methamphetamine-treatments were switched to 3 h before any testing.

3.3. Effects of methamphetamine

3.3.1. Probabilistic learning task—Neither chronic methamphetamine treatment nor genotype affected accuracy in the PLT (genotype: F(1,48) = 0.229, $p = 0.635$; drug: F (1,48) = 1.703, $p = 0.198$; Fig. 4A). methamphetamine did, however, tend to decrease (i.e., speed) latency to make a correct response in methamphetamine-treated mice (F(1,48) = 3.111, $p = 0.084$), irrespective of genotype (F(1,48) = 0.467, $p = 0.497$; Fig. 4B). Latency to retrieve rewards after correct responses was not affected by genotype (F (1,48) = 0.024, $p = 0.877$) or methamphetamine exposure (F(1,48) = 0.881, $p = 0.353$; Fig. 4C).

3.3.2. Progressive ratio breakpoint task—When mice were assessed at Day 25, neither genotype (F(1,48) = 1.224, $p = 0.274$) nor drug (F(1,48) = 0.649, $p = 0.425$) affected breakpoint in the PRBT (Fig. 4D). Chronic methamphetamine exposure in iTat mice was associated with a non-significant increase in time to respond (F(1,48) = 1.905, $p = 0.174$; Fig. 4E). Neither iTat (F(1,48) = 0.051, $p = 0.823$) nor methamphetamine (F(1,48) = 1.156, $p = 0.288$) altered the latency to retrieve rewards (Fig. 4F).

3.4. Effects of doxycycline-induced Tat expression post-methamphetamine

3.4.1. Probabilistic learning task—After withdrawal from methamphetamine and induction of Tat expression, mice showed no differences in accuracy in the PLT, either by genotype (F(1,48) = 0.188, $p = 0.667$) or by previous methamphetamine exposure (F(1,48) = 0.812, $p = 0.372$; Fig. 5A). Additionally, neither genotype (F(1,48) = 1.784, $p = 0.188$) nor methamphetamine exposure (F(1,48) = 1.255, $p = 0.268$) affected latency to make correct responses (Fig. 5B). Interestingly, both Tat expression (F(1,48) = 5.548, $p = 0.023$) and methamphetamine withdrawal (F(1,48) = 6.119, $p = 0.017$) affected latency to retrieve reward – an indirect measure of motivation. In particular, we observed a genotype ×

methamphetamine interaction (F(1,48) = 4.599, $p = 0.037$; Fig. 5C), wherein saline-treated iTat mice exhibited a slowed latency relative WT, which was rescued in iTat mice exposed to methamphetamine.

3.4.2. Progressive ratio breakpoint task—After withdrawal from methamphetamine, Tat expression in the absence of methamphetamine exposure non-significantly reduced breakpoint (F(1,48) = 1.925, $p = 0.172$; Fig. 5D). No changes, however, were detected in reaction time (genotype: F(1,48) = 0.335, $p = 0.566$; drug: F(1,48) = 0.000, $p = 0.988$; Fig. 5E) or latency to retrieve rewards (genotype: F(1,48) = 0.290, $p = 0.593$; drug: F(1,48) = 0.010, $p = 0.919$; Fig. 5F).

3.5. Within-session analysis of %correct across testing timepoints

Data were analyzed within blocks of 10 trials across 6 blocks to examine for change in %Correct during this time.

a. *During baseline*, a trend toward a significant TrialBlock effect was observed (F(5,285) = 2.0, $p = 0.081$), with no interaction with gene (F(5,285) = 0.8, $p = 0.582$). Tukey post hoc analyses revealed that trialblock 5 differed significantly from 3 and 6 ($p < 0.05$), likely simply as a low %correct during trialblock 5 (Fig. 6A).

b. *During methamphetamine treatment* before DOX, no effect of Trialblock was observed (F(5,255) = 1.3, $p = 0.280$), nor was there a Trialblock* gene interaction (F(5,255) = 0.4, $p = 0.849$), nor a Trialblock*gene* methamphetamine interaction (F (5,255) = 0.8, $p = 0.533$). A Trialblock*methamphetamine interaction was observed however (F(5,255) = 2.1, $p = 0.065$), with Tukey post hoc analyses revealing that saline-treated mice performed better than methamphetamine-treated mice in trialblock 1 ($p < 0.01$), and tended to in trialblock 3 ($p < 0.1$). Furthermore, while no difference in performance was observed in the saline-treated group given their consistently higher performance than the methamphetamine-treated mice, the latter improved over time as evidenced by higher performance in trialblocks 4, 5, and 6 vs. 1 (ps < 0.05; see Fig. 6B).

c. *Post-methamphetamine/DOX* %correct performance analysis (Fig. 6C), did reveal a main effect of TrialBlock (F(5,170) = 2.8, $p < 0.05$), with a trend toward a Trialblock*gene interaction (F (5,170) = 2.0, $p = 0.075$), a Trialblock*methamphetamine interaction (F(5,170) = 2.0, p = 0.088), with no Trialblock* gene *methamphetamine interaction (F(5,170) = 0.8, $p = 0.585$). Tukey post hoc analyses revealed that Tat- mice exhibited significantly better performance in trialblock 6 vs. 1 ($p < 0.05$), with a tendency at 4 vs. 1 ($p < 0.1$), indicative of learning in these mice. In contrast, Tat+ mice exhibited better performance during trialblocks 3 and 4 vs. 1 ($p < 0.05$), but for some reason worsened to chance levels in trialblocks 5 and 6. Interestingly, while saline-treated mice did not differ in any trialblock, methamphetamine-treated mice exhibited better performance in all trialblocks vs. 1 ($p < 0.05$). Despite

this apparent learning however, no overall difference between genotype or drug groups was observed ($F(1,34) = 0.1$, $p = 0.795$).

d. *Secondary analyses of decision-making within the PLT*. The impact of gene, methamphetamine, and their potential interactions was examined on metrics of reward- (win-stay), and punishment- (lose-shift), sensitive measures contributing to learning in the PLT. No main or interactive effects were observed across any session in the PLT however (see Supplemental Table 1 for statistics and Table 2 for means ± S.E.M.).

3.6. Effects of Tat and methamphetamine on levels of calbindin expression

No difference in calbindin expression or methamphetamine treatment was observed between groups in either the prefrontal cortex (gene: $F(1,45) = 0.088$, $p = 0.768$; drug: $F(1,45) = 0.660$, $p = 0.421$; Fig. 7A), hippocampus (gene: $F(1,45) = 1.350$, $p = 0.251$; drug: $F(1,45) = 0.077$, $p = 0.783$; Fig. 7B), or nucleus accumbens (gene: $F(1,45) = 0.050$, $p = 0.824$; drug: $F(1,45) = 0.585$, $p = 0.448$; Fig. 7C). In the ventral tegmental area, however, gene appeared to influence calbindin expression (gene: $F(1,45) = 5.203$, $p = 0.027$; Fig. 7D), with the iTat showing increased expression. However, there was no effect of drug on calbindin expression in the ventral tegmental area (drug: $F(1,45) = 2.162$, $p = 0.148$), nor was any genotype*methamphetamine interaction ($F(1,45) = 0.889$, $p = 0.351$; Fig. 7D).

4. Discussion

Here, we used mice with inducible HIV-relevant Tat protein and a recently established binge-like model of methamphetamine (methamphetamine) exposure to investigate the individual and combined effects of HIV and methamphetamine use on reward learning and motivated behavior. We found that mice with inducible Tat protein displayed no differences from control mice in either the probabilistic learning task (PLT) or the progressive ratio breakpoint task (PRBT) prior to Tat induction. Additionally, chronic methamphetamine exposure prior to the expression of the Tat impaired initial probabilistic learning of mice, without affecting effortful motivation. Interestingly, after sufficient induction of Tat with doxycycline (DOX), these mice exhibited a slowed latency to retrieve rewards (an indirect measure of motivation), in the PLT. Furthermore, withdrawal from methamphetamine appeared to mitigate this Tat effect on reward latency. When motivation was assessed directly in the PRBT, however, neither Tat nor methamphetamine exerted any effects. Finally, investigation into the potential molecular underpinnings of the effects of methamphetamine and Tat revealed an increase in calbindin expression in the VTA in iTat mice, without a drug effect.

We show here no differences in operant learning between iTat and WT mice *prior* to protein induction with DOX, consistent with previous reports [25,29]. Furthermore, mice exposed to methamphetamine exhibited impaired operant performance (FR1), during the treatment regimen. Even allowing for sufficient clearance time, methamphetamine exposure impaired initial probabilistic learning without affecting effortful motivation prior to Tat expression. While probabilistic learning has not explicitly been explicitly reported in methamphetamine users, general learning measures are impaired in former users and after chronic treatment

in rodents [53]. The lack of methamphetamine effect on motivation was surprising however, since previous research has indicated both an increase [3] and a decrease [33] in mouse motivated behavior after methamphetamine exposure. Methamphetamine-induced increases in motivation were observed shortly after administration (4 mg/kg, averaged over 4 days; [3], while we observed no effect. Our experimental design assessed mice after several methamphetamine exposures and hours later however, instead of testing them on our measure of motivation while methamphetamine was on-board. Inversely, Kitanaka et al. [33] administered a single dose of methamphetamine (1.0 mg/kg) to mice and found that it negatively affected motivation to run on a running wheel nearly 10 h later. These findings, while producing contrary conclusions, demonstrate the variability in methamphetamine pharmacodynamics over time, resulting in differential behavioral outcomes in goal-directed behavior [32]. These data are supported here partially, where methamphetamine reduced responding in mice when testing occurred while methamphetamine was on-board (e.g., Day 2 in Fig. 1), but not after a 4-day washout period (e.g., Day 6 in Fig. 1). It remains possible that the chronic nature of our methamphetamine administration model may have produced compensatory effects over time, resulting in a loss of phenotypic differences when mice were tested *after* the end of the methamphetamine regimen ("during-METH"; [30]. Additionally, during-methamphetamine testing occurred concurrently with DOX administration for Tat induction. DOX is known to act protectively in dopaminergic cells [61], which also may have attenuated the late effects of methamphetamine exposure (discussed below).

After Tat was sufficiently expressed (7 consecutive days of DOX injections; [31], mice were tested for the third, and final, time in the PLT and PRBT. While we observed no direct effect of Tat on direct measures of reinforcement learning (albeit slightly different time periods of improved PLT vs. timeblock 1, see Fig. 5), or motivated behavior, we found that iTat mice not previously exposed to methamphetamine exhibited slower latencies to retrieve the liquid reward (an indirect measure of motivation), after making a correct response in the PLT. Previous methamphetamine exposure, however, appeared to block Tat effects as these mice exhibited control levels of reward latencies. Previous studies into the effects of HIV proteins on goal-directed behavior elicited mixed results, with some indicating improved cognitive function [29], and others demonstrating decreased functions like decision-making [19,42]. This cohort of mice exhibited deficient prepulse inhibition (PPI), as did people with HIV [57]. That this PPI deficit was only observed after DOX-mediated Tat-induction support functional Tat induction in the present findings. Kesby and colleagues (2014), observed that while expression of the gp120 glycoprotein did not affect motivation for a food reward in mice, there was an increased motivation to acquire methamphetamine. In contrast to such gp120 studies, reduced motivation for drug (cocaine) or natural (sucrose) in HIV-relevant animal model (transgenic rats without active viremia expressing HIV proviral DNA, thus modeling latent HIV infections) was observed [4]. These findings could indicate that altered motivation in HIV rodent models is more apparent in certain reward contingencies (drug and simple movement) but not others (effortful motivation for food). Additionally, gp120 and TAT may interact to exert neurotoxic effects [46], which may be in-part why we did not observe WT-specific effects in this study.

It is important to distinguish between the context of the direct and secondary measure of motivated behavior. In the PRBT, mice only had to nose poke into a single illuminated hole a given number of times to earn the reward (effortful motivation). In the PLT, however, the mice were tasked with choosing between 2 different illuminated holes, one of which *almost* always produced a reward. Thus, simple motivation is more repeatedly observed in the PLT given the higher number of trials and the requirement to encode associations correctly to gain more rewards in the future. This interpretation implies that iTat mice exhibit impaired motivated behavior, but only when the task has high constant effort vs. individual effortful motivation. Future studies should alter the difficulty of the breakpoint paradigm by including visual or auditory distractors, or utilizing an effort-based decision making task, requiring choices between low and high efforts for varying rewards.

After the final round of behavioral testing, the brains of all of the mice were taken for molecular investigation. Specifically, we used immunostaining to assess the number of calbindin (CB)-expressing neurons in 4 different brain regions: the prefrontal cortex, the hippocampus, the nucleus accumbens, and the ventral tegmental area. Previous investigations have shown a loss of both CB- and parvalbumin-expressing interneurons in the post-mortem brains of people with HIV-associated encephalopathy, which was exacerbated in those who had a history of methamphetamine use [35]. Additionally, in vitro studies using mouse hippocampal neurons revealed a selective loss of CB interneurons after exposure to both Tat protein and methamphetamine [36]. Evidence demonstrates that CB interneurons can be damaged in frontal regions and the hippocampus by both Tat expression [41] and methamphetamine exposure [34], individually (reviewed in [23]. Paradoxically, we observed an increase in CB-expressing cells, specifically in the VTA of iTat mice, regardless of methamphetamine exposure. This finding is particularly interesting as GABA cells in the VTA have been posited to mediate stress-induced apathy in rodents [5,39]. This observation, combined with the proposed role of CB interneurons in regulating DAT function [7], suggests that CB upregulation after Tat expression possibly mitigates behavioral deficits associated with methamphetamine withdrawal. Alternatively, since this study utilized a DOX-inducible Tat expression mouse model, it may also be the case that CB interneurons were upregulated after DOX exposure via mechanisms relating to DAT homeostasis (discussed below).

While our results corroborate with and contribute to previous reports, several limitations may render interpretations more difficult. It is important to note that methamphetamine is an appetite suppressant [17,45,6], which could have influenced the home cage feeding behavior in the mice. Mice were caged with a maximum of 4 conspecifics, with methamphetamine- and saline-treated mice mixed. It was, therefore, difficult to measure the amount of food and water ingested by each mouse throughout the methamphetamine regimen, preventing us from determining group differences. Any differences in normal feeding behavior could have positively influenced the food seeking behavior in the methamphetamine treated mice, masking deficits. Furthermore, only male mice were tested in these behaviors, and while these mice exhibit impaired PPI consistent to people with HIV [57], it is important for future studies to include females.

To express the Tat protein, mice were injected with DOX for 7 consecutive days [31]. DOX can exert protective effects, specifically buffering tissue against apoptosis and ischemia [51,60]. Additionally, the inflammatory response associated with neurodegenerative processes is activated primarily by microglia, the macrophages of the central nervous system (reviewed in [2]. Besides inflammatory processes, microglia are relevant to HIV. Firstly, microglia express D1 and D2 dopamine receptors [14], and are, therefore, relevant to the dopamine dysregulation hypothesis of HIV-induced neurodegeneration. Microglia also act as reservoirs for HIV, potentially contributing to the development of HIV-associated neurocognitive disorders (reviewed in [55,56]. Recently, we demonstrated that gp120 transgenic mice exhibit neuroinflammation as measured by activated microglia [58], it will be important for future studies whether other HIV models such as the Tat mice exhibit similar elevated levels and if affected by substances of abuse such as methamphetamine. As discussed above, DOX appears to protect dopaminergic neurons through downregulation of microglial activity [61]. This downregulation of immune responses could attenuate the effects of Tat, which may indicate that Tat alone could have a much larger effect on goal-directed behavior than indicated in this study.

In contrast to our hypothesis, previous methamphetamine exposure appeared to have a protective effect in iTat mice. Thus, we may speculate that people with HIV may use methamphetamine as a self-medication to improve motivation, potentially via assuaging the neurodegenerative effects of the virus, an idea that has been suggested previously [50]. Future studies, therefore, should explore the effects of methamphetamine exposure *after* induction of the Tat protein to determine whether there is a direct interaction between methamphetamine and Tat.

In summary, expression of the HIV-associated Tat protein slowed time to retrieve reward (an indirect measure of reduced motivation), in the PLT assessment of reinforcement learning, which was not seen in mice with prior methamphetamine treatment. In contrast however, Tat did not decrease breakpoint (a direct measure of motivation), suggesting the impact of Tat expression may be subtle. Furthermore, methamphetamine treatment negatively affected initial probabilistic learning, without interactive with Tat expression. Dysregulation of the dopaminergic signaling in the VTA due to an increase in CB-expressing neurons in iTat mice could underlie these effects. Future assessment of Tat/methamphetamine interactions will investigate their influence on other domains of cognitive function and their associated molecular markers.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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Data Availability

Data will be made available on request.

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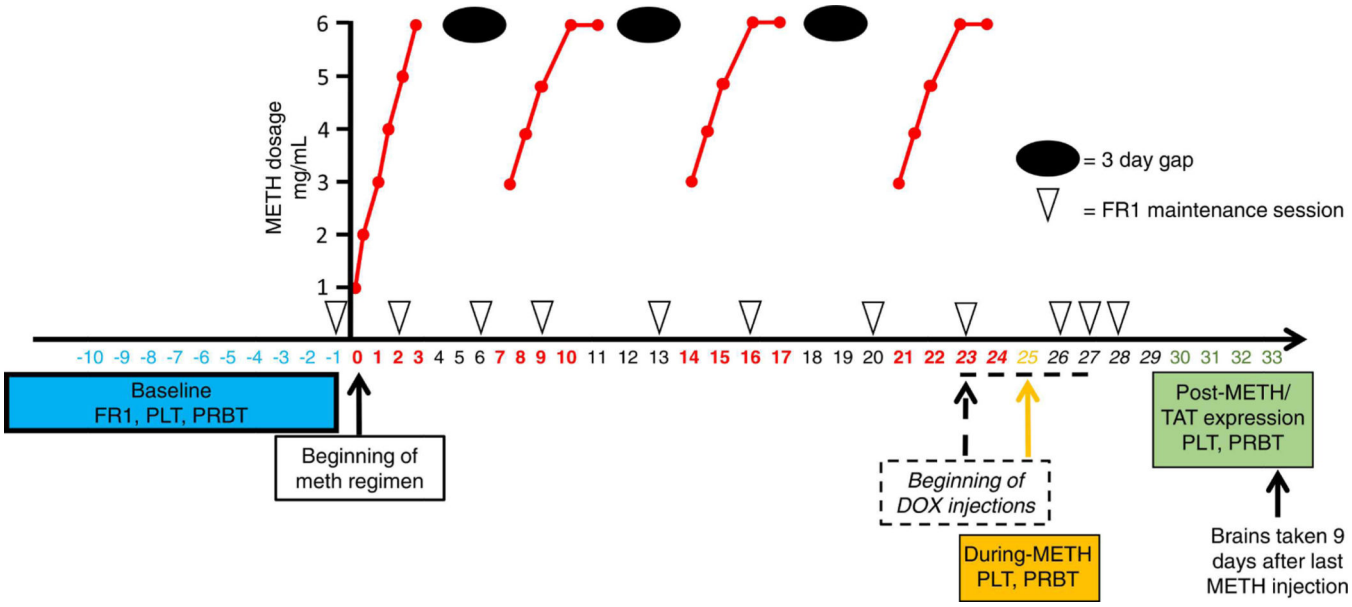

Fig. 1.

Timeline of methamphetamine (METH) exposure and Tat expression. Prior to methamphetamine exposure, mice were trained to nose poke in illuminated holes in an operant chamber (FR1) and were subsequently tested in the Probabilistic Learning Task (PLT) and Progressive Ratio Breakpoint Task (PRBT). During the methamphetamine exposure period, mice were administered drug or saline control four times per day (10:00, 12:00, 14:00, and 16:00). Initial methamphetamine administration was low (1 mg/mL) and steadily increased to a higher dose (6 mg/mL) over four days. During subsequent exposure days, the 10:00 dose was set at 3 mg/mL on the first day and increased to 6 mg/mL by the third day. Mice were tested on FR1 during the third day of each exposure regimen to maintain responding. Each of the four exposure blocks was separated by a 3-day period where mice did not receive injections and were provided additional FR1 training to maintain responding. 24 days after the initial methamphetamine exposure, mice were treated with 100 mg/kg doxycycline for 7 days and retested on the PLT and the PRBT. After the 7-day doxycycline exposure, mice were tested for the final time on the PLT and PRBT, after which point mice were sacrificed and their brains collected for molecular analysis (9 days after last methamphetamine exposure).

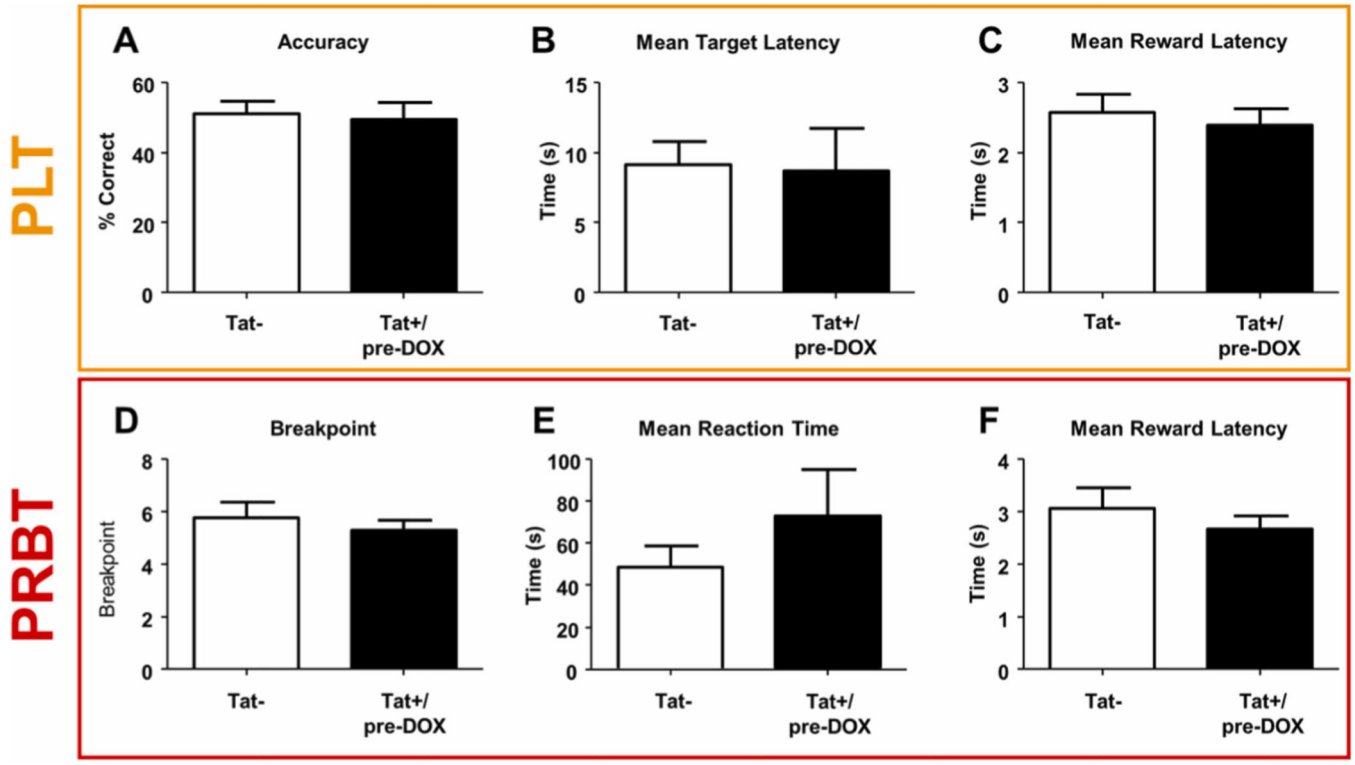

Fig. 2.

Mice possessing the Tat promotor transgene perform no different from their wildtype (Tat-) littermate mice. Both genotypes performed at chance during baseline PLT (A). Both groups of mice exhibited similar latencies to make a correct response (B) and retrieve rewards (C). In the PRBT, both Tat- and Tat+ mice had similar breakpoints (D) and displayed similar reaction times (E) and reward latencies (F). Data presented as mean + SEM.

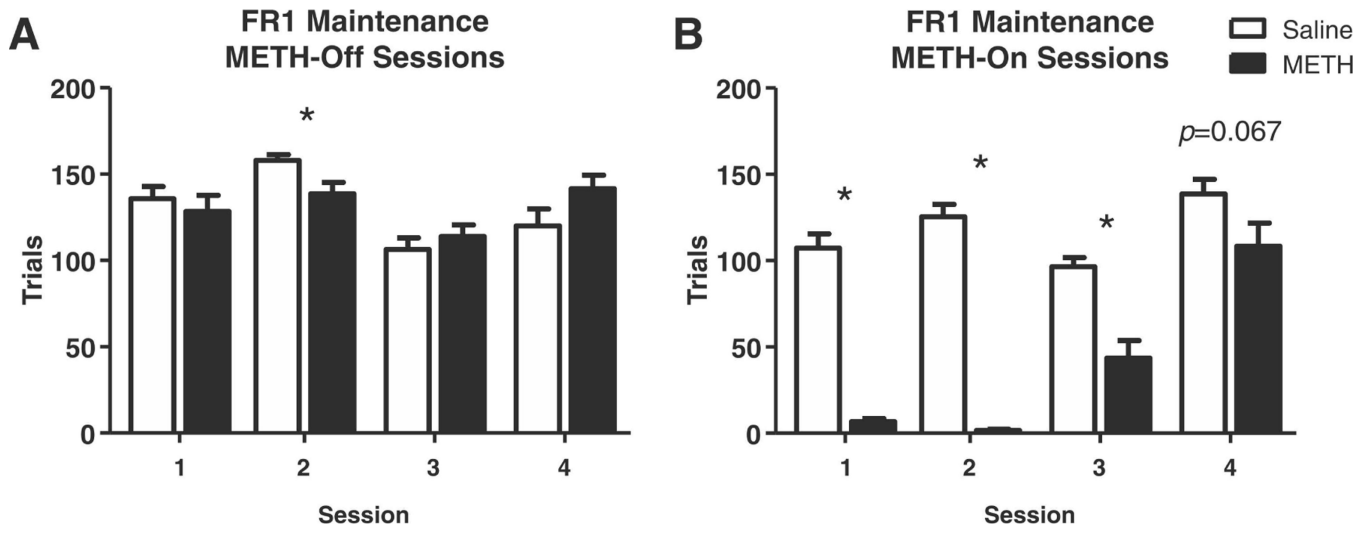

Fig. 3.

Methamphetamine (METH) exposure reduces responding in FR1 maintenance sessions. Across genotypes, mice in both drug groups performed similarly in the absence of METH (A). METH exposure, however, decreased responding across all maintenance sessions, with the strongest effect seen in sessions closest to METH treatment (B). Data presented as mean ± SEM. * denotes $p < 0.05$ vs. saline within each testing session.

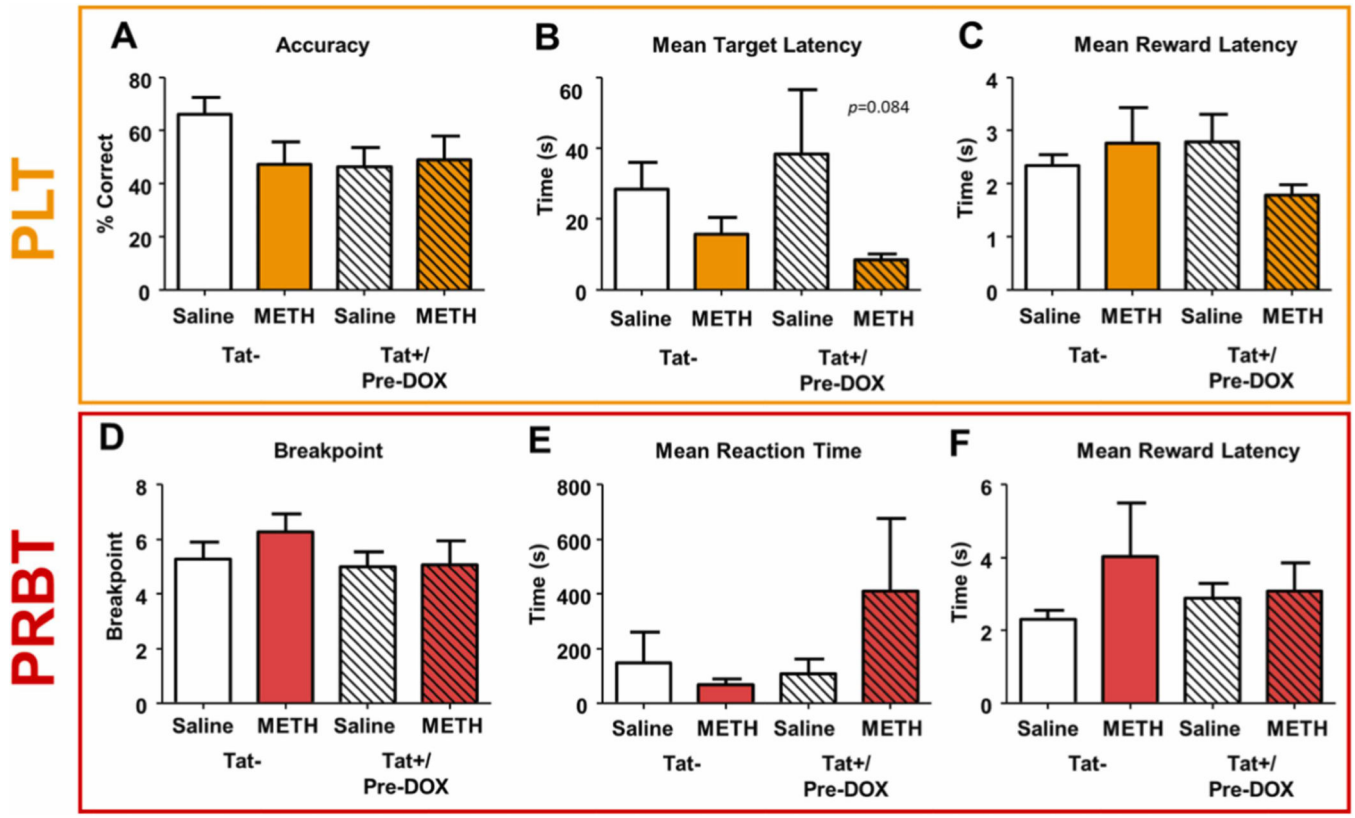

Fig. 4.

Chronic methamphetamine (METH) exposure does not affect overall reward learning or motivation. In the PLT, neither genotype nor methamphetamine exposure affected overall learning performance (despite higher scores in saline-treated mice; A), latency to make correct responses (B), or latency to retrieve rewards (C). Additionally, genotype and methamphetamine did not affect breakpoint (D), reaction time (E), or reward latency (F) in the PRBT. Data presented as mean ± SEM.

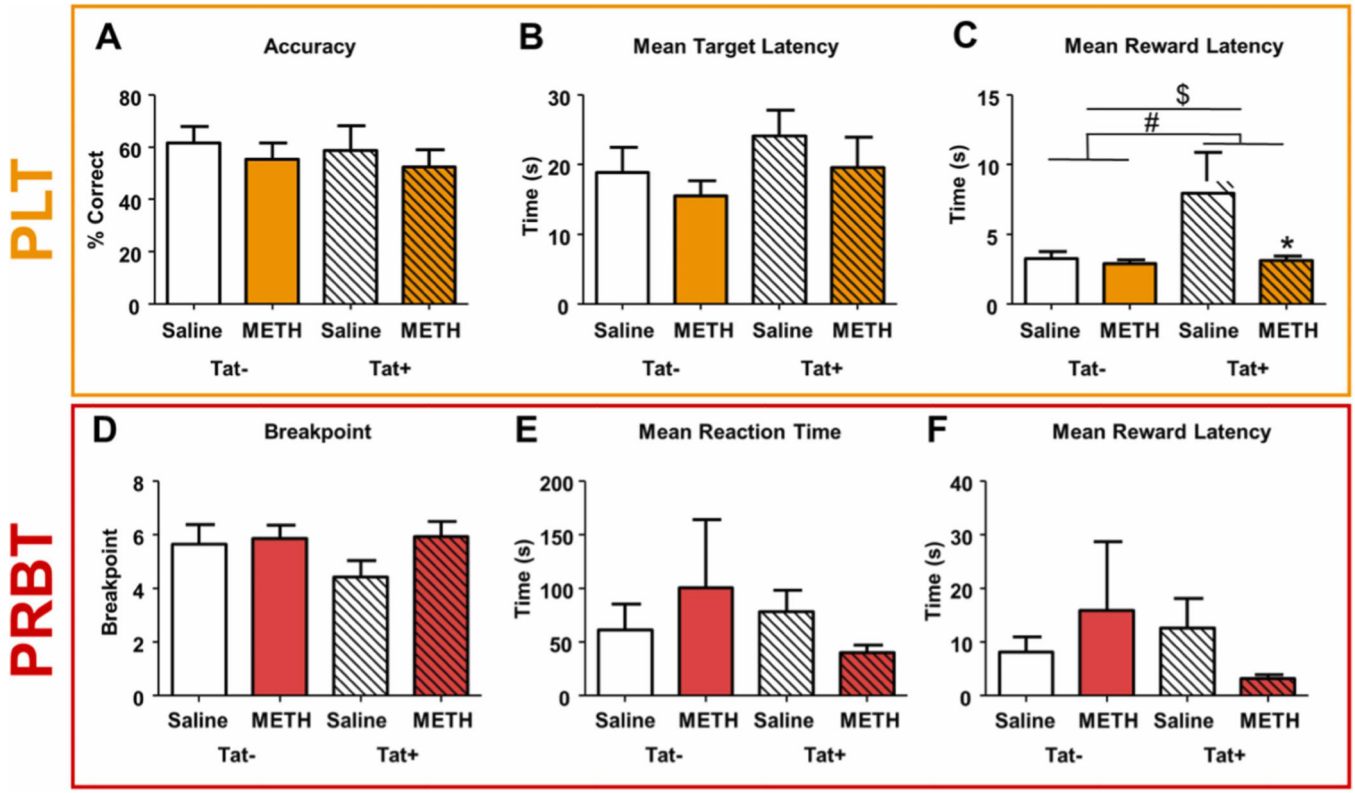

Fig. 5.

Tat induction from doxycycline (DOX), impairs a secondary measure of motivation, rescued by previous chronic methamphetamine (METH) exposure. Genotype and methamphetamine exposure did not affect accuracy (A) or target latency (B) in the PLT. Tat expression did slow latency to retrieve rewards however (C), which was rescued by previous METH exposure. Additionally, neither genotype nor drug exposure affected breakpoint (D), reaction time (E), or reward latency (**F**) in the PRBT. Data presented as mean ± SEM. * = $p < 0.05$ vs. Saline; $p < 0.05$. # = main effect of genotype; $p < 0.05$. $ = main effect of drug; $p < 0.05$.

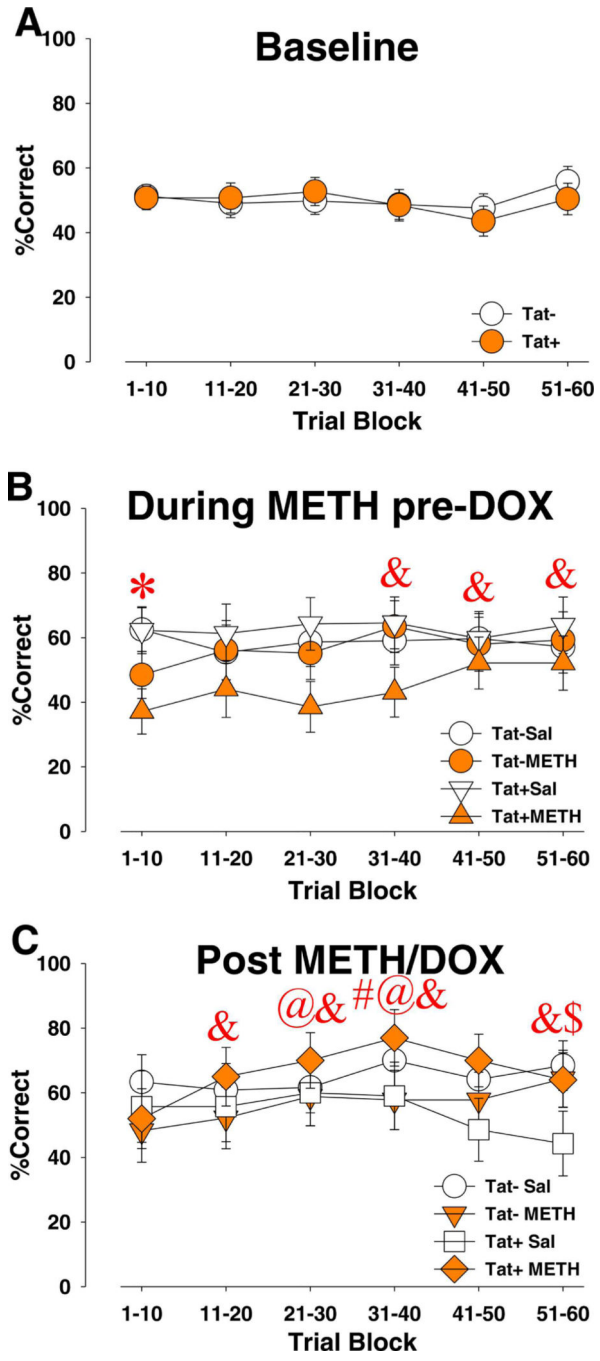

Fig. 6.

Limited probabilistic learning impaired by methamphetamine treatment. %Correct performance of mice across trial blocks during baseline (A), methamphetamine (METH) treatment (B), and after METH and Tat induction via doxycycline (DOX; C). These data reveal that mice exhibit some within-session learning of the probabilistic learning task that is stronger with repeated testing. Importantly, both during- and post-METH treatment revealed that initial performance was worse in METH-treated mice but that improved to saline-treated levels over trials blocks. Data presented as mean ± S.E.M. * = $p < 0.05$

vs. methamphetamine-treated mice. & = $p < 0.05$ vs. trialblock 1–10 in methamphetamine-treated mice. @ = $p < 0.05$ vs. trialblock 1–10 in Tat+ mice. $ = $p < 0.05$ vs. trialblock 1–10 in Tat- mice irrespective of treatment. # = $p < 0.1$ vs. trialblock 1–10 in Tat- mice irrespective of treatment.

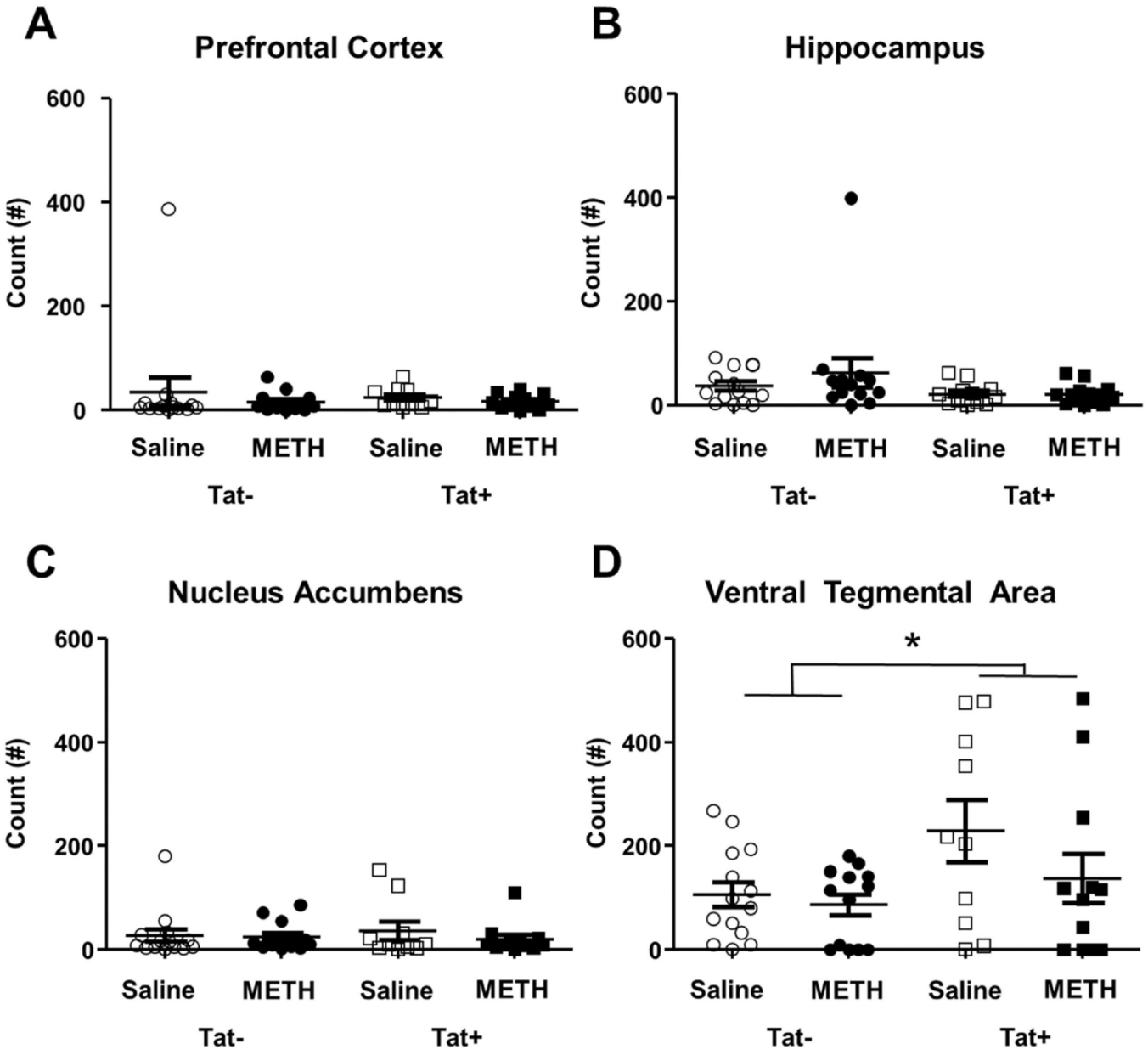

Fig. 7.

Tat expression increases calbindin expression in the ventral tegmental area. Neither genotype nor drug exposure altered calbindin expression in the prefrontal cortex (A), hippocampus (B), or nucleus accumbens (C). In the ventral tegmental area, Tat expression increased calbindin expression, not otherwise influenced by prior methamphetamine (METH) exposure (D). Data presented as individual data points as well as mean ± S.E.M., * denotes $p < 0.05$ in Tat+ vs. Tat- mice after doxycycline treatment to both groups.