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Publication Date

2023

DOI

10.1016/j.pbb.2022.173499

Peer reviewed

HHS Public Access

Author manuscript

Pharmacol Biochem Behav. Author manuscript; available in PMC 2024 January 01.

Published in final edited form as:

Pharmacol Biochem Behav. 2023 January ; 222: 173499. doi:10.1016/j.pbb.2022.173499.

iTat transgenic mice exhibit hyper-locomotion in the behavioral pattern monitor after chronic exposure to methamphetamine but are unaffected by Tat expression

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Abstract

Although antiretroviral therapy (ART) has increased the quality of life and lifespan in people living with HIV (PWH), millions continue to suffer from the neurobehavioral effects of the virus. Additionally, the abuse of illicit drugs (methamphetamine in particular) is significantly higher in PWH compared to the general population, which may further impact their neurological functions. The HIV regulatory protein, Tat, has been implicated in the neurobehavioral impacts of HIV and is purported to inhibit dopamine transporter (DAT) function in a way similar to methamphetamine. Thus, we hypothesized that a combination of Tat expression and methamphetamine would exert synergistic deleterious effects on behavior and DAT expression. We examined the impact of chronic methamphetamine exposure on exploration in transgenic mice expressing human Tat (iTat) vs. their wildtype littermates using the behavioral pattern monitor (BPM).

During baseline, mice exhibited sex-dependent differences in BPM behavior, which persisted through methamphetamine exposure, and Tat activation with doxycycline. We observed a main effect of methamphetamine, wherein exposure, irrespective of genotype, increased locomotor activity and decreased specific exploration. After doxycycline treatment, mice continued to exhibit drug-dependent alterations in locomotion, with no effect of Tat, or methamphetamine interactions. DAT levels were higher in wildtype, saline-exposed males compared to all other groups.

These data support stimulant-induced changes of locomotor activity and exploration, and suggest that viral Tat and methamphetamine do not synergistically interact to alter these behaviors in mice.

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These findings are important for future studies attempting to disentangle the effect of substances that impact DAT on HAND-relevant behaviors using such transgenic animals.

Keywords

iTAT HIV model; Behavioral pattern monitor; Mouse; Movement; Exploration; Dopamine

1. Introduction

The Joint United Nations Programme on HIV/AIDS estimates approximately 76 million people have been infected with HIV since the beginning of the epidemic (UNAIDS, 2020). Though successful antiretroviral therapy (ART) has reduced viral progression and mortality in people living with HIV (PWH), HIV-associated neurocognitive disorder (HAND) persists (Wang et al., 2020). Further, PWH use illicit drugs (e.g. methamphetamine) at higher rates than the general population (Clark et al., 2012; Mitchell et al., 2006), which are also known to impair cognitive and motor performance. Thus, it has been proposed that drug use may interact with the HIV virus to produce synergistic effects on physiology and behavior (see Mediouni et al., 2015; Reiner et al., 2009 for reviews). Indeed, cocaine use exacerbated response inhibition deficits (Wakim et al., 2022), and previous methamphetamine use disorder was associated with worsened sensorimotor and memory deficits (Walter et al., 2021a; Walter et al., 2021b) in PWH.

Transgenic rodents expressing HIV-relevant genes have physiological and behavioral phenotypes akin to PWH, and are invaluable tools for determining the behavioral impact of substance use and HIV, and contributing mechanisms. Specific proteins relevant to HIV have been associated with neurobehavioral changes, particularly the envelope protein, gp120, and the regulatory protein, Tat (see Gaskill et al., 2017; Thaney et al., 2018 for reviews). Transgenic rodent models expressing these viral proteins have revealed that both gp120 and Tat interact with methamphetamine exposure to effect cognitive and motor deficits in mice (Baek et al., 2020; Henry et al., 2013; Kesby et al., 2016; Kesby et al., 2015; Kesby et al., 2017; Liu et al., 2014; Walter et al., 2021b). The exact mechanism by which HIV and methamphetamine interact to alter behavior however, remains unknown.

HAND deficits span motor and cognitive domains, suggesting dysfunctional dopamine (DA) signaling as a contributing factor. Indeed, cerebrospinal fluid dopamine levels are lower in PWH (Berger et al., 1994) and dopamine transporter (DAT) availability is largely decreased in deep cortical tissues integral for cognitive and motor function (i.e. basal ganglia) in both HAND (Chang et al., 2008), and HIV-associated dementia diagnosed (Wang et al., 2004) PWH. Lower DAT levels in the basal ganglia were also associated with enhanced memory and psychomotor impairments in HAND diagnosed PWH (Chang et al., 2008). Like methamphetamine, the Tat protein alters DA via the inhibition of DAT, which may contribute to their interactive effects on behavior. In fact, Appadoo et al. (2017) demonstrated the combination of Tat and methamphetamine exposure decreased DAT function further than either manipulation alone, though no behavior was assessed in

this study. Thus, DAT inhibition may be a shared mechanism by which methamphetamine exposure and the Tat protein alter synergistically alter behavior.

Locomotor activity and exploration are largely altered by DAT inhibition (e.g., methamphetamine exposure), and HIV protein exposure in mice, as measured in the behavioral pattern monitor (BPM) task (Henry et al., 2013; Kwiatkowski et al., 2019; van Enkhuizen et al., 2014; Young et al., 2010b). Given the importance of activity to measuring cognitive performance, here, we used the inducible-Tat (iTat) transgenic mouse model (Kim et al., 2003) to determine the individual and combined effects of doxycycline-induced Tat expression and chronic methamphetamine exposure on locomotor and exploratory activity in the BPM. The iTat model was chosen as it produces Tat expression in the brain similar to levels observed in the brains of PWH, and recreates physiological and behavioral phenotypes relevant to HIV viral progression, including, impaired astrocyte proliferation and neurogenesis, as well as cognitive and motor impairments (Langford et al., 2018). Additionally, unlike congenital models (e.g., gp120 mice), the protein expression in the iTat model can be induced at time points more consistent with human HIV onset, which can clarify the impact of Tat at specifically-timed experimental manipulations (i.e. following drug exposure). We further assessed the long-term effects of methamphetamine exposure and Tat expression on DAT expression via immunostaining. We hypothesized that, while both methamphetamine and Tat alone would alter BPM measures, their combined effects would synergistically alter activity and exploration. Additionally, we hypothesized that iTat mice exposed to methamphetamine would exhibit reduced numbers of DAT-positive cells in brain regions associated with locomotion and exploratory behavior.

2. Methods

2.1. Animals

Male and female adult mice (Baseline Experiment: 30 WT females/31 WT males, 29 iTat females/29 iTat males; Post- methamphetamine Experiment: 30 WT females/30 WT males, 29 iTat females/29 iTat males; Post-induced TAT expression Experiment: 30 WT females/27 WT males, 28 iTat females/24 iTat males; immunohistochemistry: 28 WT females/29 WT males, 22 iTat females/28 iTat males) on a C57Bl/6 J background were housed one-to-four mice per cage and maintained in a temperature-controlled (21 ± 1 °C) vivarium on a 12 h/12 h reversed light-dark cycle (1900 Lights On, 0700 Lights Off). Mice were 14 weeks old at the beginning of testing. Varied housing levels were a result of severe cage-mate aggression (single housing was in the vast minority). Mice lacking Tat (WT) contained GFAP promoter-controlled Tet-binding protein ($TGFAP^+$) while mice expressing Tat (iTat) contained $TGFAP^+$ promotor and the TRE promotor-Tat protein transgene ($TAT86^+$). All mice were supplied by Dr. Marcus Kaul (UC Riverside) 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 at least one week before the start training. Testing occurred in the dark phase (1300 to 1800). 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

Methamphetamine was administered following previously described dosing regimens (Kesby et al., 2018a; Kesby et al., 2019; Kesby et al., 2018b), as this method fits models of total use per day per month in humans. Briefly, mice were administered subcutaneous injections of either 0.9 % saline or methamphetamine hydrochloride (5 ml/kg; Sigma, St. Louis, MO, USA) for 25 days (4-day block of injections separated by 3 days of washout; 4 injections/day at 1200, 1400, 1600, and 1800). Drug concentrations were gradually increased over four day injection blocks to produce binge-like exposure (Fig. 1).

Beginning on Day 24 of the methamphetamine regimen, mice were given intraperitoneal injections of 100 mg/kg doxycycline (DOX; doxycycline hyclate; Sigma, St. Louis, MO, USA) daily for 7 days (days 24–30), paired with the first daily injection of the methamphetamine regimen (i.e., at 12:00), consistent with previous studies (Walter et al., 2021b) (Fig. 1). DOX was used to induce Tat expression in the iTat mice, while having no effect in WT mice, to assess the effects of Tat 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. Behavioral pattern monitor

The mouse BPM, used to record animal movement and exploratory behavior, consisted of a Plexiglas chamber (area: 30.5 × 61 × 38 cm) illuminated by a single light source located above the arena (Kwiatkowski et al., 2019; Risbrough et al., 2006; van Enkhuizen et al., 2013). The chamber contained 3 floor holes and 8 wall holes and was equipped with 12 × 24 infrared photobeams 1 cm above the floor (2.5 cm apart) to detect holepoking behavior. A set of 16 infrared photobeams located 2.5 cm above the floor was used to detect rearing behavior. Mouse location was recorded every 0.1 s with its position defined across nine unequal regions (center, 4 corners, 4 walls). At the beginning of each session, mice were placed at the top left-hand corner of the chamber, facing the corner. Activity was measure using the 12 × 24 infrared photobeams 1 cm above the floor (2.5 cm apart). Behavioral measures have been defined previously (Cope et al., 2021) and were recorded during a 30 min session.

The primary outcomes measured included total behavioral activity (total number of distinct behaviors during testing; “counts”), transition behavior (crossing from one predefined area to another), travel distance (total distance traveled in cm), holepoking (total number of pokes across all 11 holes), rearing (number of movements in the y-axis), and spatial d (index of movement linearity with values closer to 1 reflecting straighter path movement and values closer to 2 indicating highly circumscribed movement; Paulus and Geyer, 1991). Testing occurred: 1) at baseline prior to drug administration 2) on day 23 at least 1 h following METH exposure and 3) on day 32 following TAT expression (Fig. 1).

2.4. Histology

Mouse brain hemispheres were collected one day following the last BPM test and fixed for 24 h in 4 % paraformaldehyde and embedded in paraffin. Briefly, as previously described (Kesby et al., 2018b), mouse hemibrain tissue sections (5 μm thickness) 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, dewaxed 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 im- munoreactivity 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.

2.5. Statistical analyses

All statistical analyses were performed using IBM SPSS Statistics 27 (Armonk, NY, USA). Behavioral and molecular data were analyzed using mixed analysis of variance (ANOVA), with drug and genotype as between-subjects factors. Results are expressed as mean ± standard error of the mean (SEM). Differences were considered statistically significant at $p < 0.05$.

3. Results

3.1. BPM baseline

Mouse movement and exploration (primary measures: behavioral counts, transitions, total distance traveled, hole pokes, rearing, and spatial d) were measured in the BPM.

During Baseline, mice exhibited a main effect of sex on several measures including counts($F_{(1,111)} = 65.874$, $p < 0.001$; Fig. 2A), transitions ($F_{(1,111)} = 53.004$, $p < 0.001$; Fig. 2B), distance traveled ($F_{(1,111)} = 61.516$, $p < 0.001$; Fig. 2C), rearing ($F_{(1,111)} = 33.586$, $p < 0.001$; Fig. 2E), and spatial d ($F_{(1,111)} = 7.292$, $p = 0.008$; Fig. 2F), with female mice performing fewer of these activities and having a higher spatial d compared to males. There was no main effect of sex on holepoking ($F_{(1,111)} = 0.921$, $p = 0.339$; Fig. 2D). There were no main effects of gene (counts: ($F_{(1,111)} = 0.186$, $p = 0.667$; transitions: ($F_{(1,111)} = 0.075$, $p = 0.784$; distance traveled: $F_{(1,111)} = 0.020$, $p = 0.888$; holepoking: $F_{(1,111)} = 2.102$,

$p = 0.150$; rearing: $F_{(1,111)} = 0.417$, $p = 0.520$; spatial d: $F_{(1,111)} = 0.001$, $p = 0.974$) or drug (counts: $F_{(1,111)} = 0.001$, $p = 0.972$; transitions: $F_{(1,111)} = 0.013$, $p = 0.908$; distance traveled: $F_{(1,111)} = 0.001$, $p = 0.970$; holepoking: $F_{(1,111)} = 0.000$, $p = 0.983$; rearing: $F_{(1,111)} = 0.883$, $p = 0.350$; spatial d: $F_{(1,111)} = 0.372$, $p = 0.543$). The lack of main effects are important given that this testing period was before DOX-induced Tat expression or methamphetamine treatment, confirming consistency before treatment. There were also no interactions between any of the groups ($ps > 0.1$).

3.2. Effects of 23-day methamphetamine binge exposure on BPM behavior in iTat mice

On day 23, at least 1 h following the first methamphetamine injection, mice were once again tested in the BPM. Across all behavioral measures, we observed a main effect of drug ($F_{(1,110)} = 225.895$, $p < 0.001$) and sex ($F_{(1,110)} = 15.044$, $p < 0.001$), with all males, and all mice previously exposed to METH, exhibiting more counts overall (Fig. 3A-F). Additionally, there was a sex × drug interaction in total counts ($F_{(1,110)} = 7.671$, $p = 0.007$; Fig. 3A). Similarly, transitions and distanced traveled showed a main effect of drug (transitions: $F_{(1,110)} = 161.555$, $p < 0.001$; Fig. 3B; distanced traveled: $F_{(1,110)} = 197.111$, $p < 0.001$; Fig. 3C), with methamphetamine-exposed mice exhibiting increased activity. There was also a main effect of sex in transitions ($F_{(1,110)} = 8.820$, $p = 0.004$) and distanced traveled ($F_{(1,110)} = 6.444$, $p = 0.013$), with males exhibiting more of each behavior. For hole poking, female mice, overall, exhibited more activity (main effect of sex: $F_{(1,110)} = 9.065$, $p = 0.003$), while, methamphetamine-exposed mice exhibited decreased holepoking (main effect of drug: $F_{(1,110)} = 128.837$, $p < 0.001$; sex × drug interaction: $F_{(1,110)} = 128.837$, $p < 0.001$; Fig. 3D). Finally, methamphetamine-exposed mice exhibited decreased rearing ($F_{(1,110)} = 89.818$, $p < 0.001$; Fig. 3E) and spatial d $F_{(1,110)} = 15.453$, $p < 0.001$; Fig. 3F) compared to control, with rearing showing an additional sex × drug interaction ($F_{(1,110)} = 7.671$, $p = 0.007$).

3.3. Effects of TAT expression and post-methamphetamine binge on BPM activity in iTat mice

Seven days after the last methamphetamine exposure and 2 days after the last DOX administration (Day 32), mice were tested in the BPM. Overall, the primary measurements of the BPM were not affected by Tat expression.

During the final assessment, methamphetamine-exposed mice and all male mice exhibited higher total counts (drug: $F_{(1,101)} = 14.600$, $p < 0.001$; sex: $F_{(1,101)} = 4.620$, $p = 0.034$; Fig. 4A), higher transitions (drug: $F_{(1,101)} = 9.319$, $p = 0.003$; sex: $F_{(1,101)} = 6.131$, $p = 0.015$; Fig. 4B), and a longer distanced traveled (drug: $F_{(1,101)} = 13.072$, $p < 0.001$; sex: $F_{(1,101)} = 5.994$, $p = 0.016$; Fig. 4C), with no interactions between any groups. Lastly, while all male mice exhibited higher rearing ($F_{(1,101)} = 14.506$, $p < 0.001$), there were no main effects or interactions in either holepoking or spatial d (Fig. 4D-F).

3.4. DAT expression after TAT expression in methamphetamine-exposed mice

There were no main effects sex ($F_{(1,99)}=0.864$, $p = 0.355$), gene ($F_{(1,99)}=0.011$, $p = 0.918$) or drug ($F_{(1,99)}=0.039$, $p = 0.562$) on DAT expression in the nucleus accumbens (Fig. 5A). While there were no significant effects in the ventral tegmental area, we observed that

methamphetamine treatment tended to be associated with reduced DAT ($F_{(1,99)}$=3.408, p = 0.068) driven primarily by higher levels in male WT saline-exposed mice (trend interactions: gene × drug: $F_{(1,99)}$=3.467, p = 0.066; sex × gene: $F_{(1,99)}$=3.029, p = 0.0858; sex × gene × drug: $F_{(1,99)}$=3.075, p = 0.083; Fig. 5B). In the caudate putamen, we observed no main effect of sex ($F_{(1,99)}$=1.357, p = 0.247), gene ($F_{(1,99)}$=0.037, p = 0.847), or drug ($F_{(1,99)}$=0.012, p = 0.912; Fig. 5C). We found, however, that gene and drug tended to interact ($F_{(1,99)}$=3.541, p = 0.063) with both female WT saline- and female iTat METH-exposed mice exhibiting higher DAT.

4. Discussion

In this study, we determined the effects of chronic methamphetamine exposure and Tat expression on activity and exploratory behavior in the behavioral pattern monitor (BPM). Utilizing both an established methamphetamine-exposure regimen (Kesby et al., 2018a; Kesby et al., 2019; Kesby et al., 2018b) and a validated mouse model of inducible Tat (iTat) expression (Langford et al., 2018), mice were assessed in the BPM 1) Before drug exposure (baseline BPM); 2) Shortly after chronic drug exposure, but before Tat induction with doxycycline (pre-DOX); and 3) After Tat induction following chronic drug exposure (post-DOX). At baseline, sex differences were observed wherein male mice exhibited higher activity and rearing-specific exploration but more linear activity as reflected by a lower spatial d. After chronic methamphetamine exposure (pre-DOX), the sex differences observed in baseline remained, while methamphetamine increased overall activity irrespective of sex, and decreased specific exploration and spatial d. After Tat induction (post-DOX), mice previously exposed to methamphetamine continued to exhibit elevated activity, but Tat expression did not affect activity, with no interaction observed. Following the post-DOX behavioral assessment, we observed modest decreases of DAT in the ventral tegmental area of methamphetamine-exposed mice, driven by saline-treated male wildtype mice.

Studies that utilize the BPM typically do not observe baseline sex differences in activity and exploration when tested in humans (Minassian et al., 2016; Perry et al., 2009), mice (Milienne-Petiot et al., 2017a; van Enkhuizen et al., 2014; van Enkhuizen et al., 2013; Young et al., 2010a, 2010b) or rats (Roberts et al., 2021). Here, we found that male mice displayed higher activity and exploration, and had simpler travelling paths than females, and these sex differences were maintained following chronic methamphetamine binge exposure. One other study from our group found male mice exhibited higher levels of activity and exploration, similar to our findings, but no changes in spatial d (Cope et al., 2021). The meaning of the sex differences are unknown but could be due to age, estrous cycle, or natural exploratory activity levels, each contributing to sex differences in activity using other paradigms e.g., the open field test (Datta et al., 2019; Simmel et al., 1976; Tran et al., 2021; van den Buuse et al., 2017). The BPM task more closely measures exploratory behavior and activity patterns however, and reduces potential predator fear relative to open field tests through the use of a closed lid apparatus. More work is therefore required to understand the meaning of any sex differences observed in the BPM.

Stimulant exposure alters each domain of motor function differently. For example, Torres et al. (2021) found methamphetamine exposed perinatal mice demonstrated hyper locomotor

activity, but no changes to motor coordination as measured by the Rotarod test. Here, male and female mice demonstrated methamphetamine-induced hyperactivity, and decreased specific exploration and spatial d following a binge exposure. This pattern was consistent with acute amphetamine treatment in mice (Minassian et al., 2016; Perry et al., 2009; Young et al., 2010a). Additionally, animals continued to display altered activity and exploration following a week of methamphetamine abstinence, consistent with long-lasting stimulant-induced behavioral sensitization (Robinson and Becker, 1986; Valjent et al., 2010). These long-lasting behavioral effects are likely modulated by the dopaminergic system as methamphetamine increases dopamine activity by activation of dopamine-1 and −2 receptors (Brown et al., 2002; Sonsalla et al., 1986), increasing activity in animals (Camp et al., 1994; Pritchard et al., 2012). The increased activity and lower spatial d observed may be modulated by the DAT as genetic and pharmacological inhibition of DAT in mice caused the same pattern of behaviors (Milienne-Petiot et al., 2017a; Perry et al., 2009; van Enkhuizen et al., 2014; van Enkhuizen et al., 2013; Young et al., 2010a), though specific exploration is increased by these manipulations as seen in human bipolar patients, rather than decreased as demonstrated here and after amphetamine. Thus, the combined effect on DAT and norepinephrine inhibition likely underlies the change in specific exploration relative to selective DAT inhibition. The hyper-active and -exploratory phenotype of DAT KD mice was modulated by dopamine (Milienne-Petiot et al., 2017a; Milienne-Petiot et al., 2017b); thus, methamphetamine effects will likely have been inpart from inhibition of DATs.

Independently, both Tat and methamphetamine inhibit DAT function (Goodwin et al., 2009; Xie and Miller, 2009; Zhu et al., 2011; Zhu et al., 2009), and alter locomotor and exploratory responding (Henry et al., 2013; Henry et al., 2011; Nass et al., 2020). When combined, exposure to methamphetamine and Tat expression synergistically interacted to further dysregulate the dopaminergic system and dopamine-related behaviors (Cass et al., 2003; Liu et al., 2014; Maragos et al., 2002). For example, methamphetamine exposure in combination with intra-striatal Tat microinjections in rats produced significantly more dysregulation to striatal dopamine levels and DAT binding capacity, compared to methamphetamine or Tat alone (Maragos et al., 2002). At a behavioral level, Tat expression in mice produced increased methamphetamine-induced reward enhancement (Kesby et al., 2016), and locomotor sensitization (Kesby et al., 2017), and chronic methamphetamine further impaired PPI of these iTat mice (Walter et al., 2021b). Unexpectedly, the expression of Tat by DOX did not alter methamphetamine-induced alterations of activity or exploration in our study. The differences between our findings and those of Kesby et al. (2016, 2017) could be attributed to methamphetamine dosing regimens, as animals in their studies were exposed acutely to methamphetamine, rather than the chronic methamphetamine exposure in our study. The expression of Tat expression has increased (Joshi et al., 2020; Nass et al., 2020) and decreased (Liu et al., 2014; Walter et al., 2021b; Zhao et al., 2020) activity in rodents, however several factors vary across studies including the route (intracranial, systemic, food), and timing (acute, chronic) of DOX administration, and the particular apparatus used to monitor activity between laboratories (open field, homecage, BPM, novelty exploration, hole-board exploration), which may have driven these inconsistent findings. Indeed, within the same study, Joshi et al. (2020) report that acute DOX exposure by injection produced no activity changes in locomotor boxes, while chronic DOX exposure

by food significantly decreased movement in the iTat mice. Some exploratory changes may occur over time with a long test session, but a within time-bin analysis over the 30 min did not reveal any findings that would suggest interaction between drug and genotype (data not shown).

Since Tat expression did not alter any BPM measures, the Tat protein likely does not synergistically nor additively contribute to the hyperlocomotor phenotype typically found in the BPM following stimulant exposure. In addition to altering behavior, the chronic methamphetamine binge exposure in our study produced subtle decreases of DAT in male mice. These findings are consistent with literature showing decreased DAT in human methamphetamine users using positron emission tomography (McCann et al., 1998; Sekine et al., 2001; Sekine et al., 2003; Volkow et al., 2001). These data are also consistent with methamphetamine-induced reductions of brain DAT in other rodent studies (Panmak et al., 2021). It should be noted that brains were collected following a 7 day washout period from the methamphetamine, and DAT expression can recover following drug abstinence (Volkow et al., 2001), therefore more robust decreases in DAT may have been observed across sex if brains were taken shortly following the methamphetamine exposure. Thus, methamphetamine-induced changes in BPM measures could relate to changes in brain DAT following stimulant exposure. The observed sex-differences could also mean that female DAT function recovers quicker than males.

In addition to the experimental limitations noted above, the iTat model in itself poses some constraints on the interpretation of our observed results. For one, the iTat model expresses one of the many HIV-1 relevant proteins (i.e. Tat) within a specific cell type (i.e. astrocytes; Kim et al., 2003; Langford et al., 2018). Thus, effects observed in this model are best understood as the specific impact of Tat-expressing astrocytes on physiological and cognitive functioning. However, while this model does not recreate the interaction of HIV-1 viral proteins found in PWH, it enables the investigation of Tat independently, helping to disentangle the effect of this specific protein on physiological and behavioral outcomes in HIV. Another caveat results from the use of doxycycline to induce Tat, as this compound has demonstrated neuroprotective properties in vivo and in vitro (see Santa-Cecilia et al., 2019 for a review), which may counteract Tat-induced neurotoxicity and relevant changes to physiology and behavior. It is therefore possible that doxycycline prevented an effect of Tat expression on BPM behavior in our study.

In conclusion, chronic binge methamphetamine exposure changed the locomotor activity, exploration, and movement patterns of mice in the BPM, consistent with previous stimulant literature. DOX-induced tat expression did not alter methamphetamine-induced changes, suggesting no effect or synergy of effects by this viral protein. Chronic binge methamphetamine exposure decreased DAT in the brains of male mice, possible contributing to the behavioral changes seen in the mouse BPM. Finally, these data indicate a lack of interactive effects by Tat and methamphetamine exposure on the basal locomotor activity in mice, suggesting that other viral proteins may contribute to such interactions, but also that studies on cognitive outcomes are unlikely to be confounded by changes in basal activity. These findings are important for future studies attempting to disentangle the impact of substance use on HAND-relevant behaviors using such transgenic animals.

Funding Acknowledgement

This work was supported by NIDA funding R01DA051295.

Data availability

Data will be made available on request.

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Day	1	2	3	4	8	9	10	11	15	16	17	18	22	23	24	25
10 AM	1	3	5	6	3	5	6	6	3	5	6	6	3	5	6	6
12 AM	1	3	5	6	3	5	6	6	3	5	6	6	3	5	6	6
2 PM	2	4	5	6	4	5	6	6	4	5	6	6	4	5	6	6
4 PM	2	4	5	6	4	5	6	6	4	5	6	6	4	5	6	6

* Injections in mg/kg

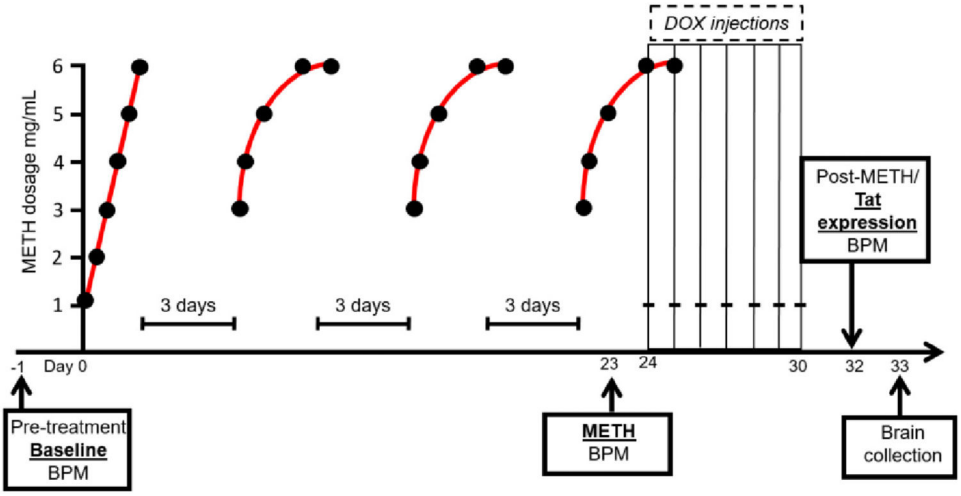

Fig. 1.

Experimental timeline. Mice were assessed on the behavior pattern monitor (BPM) prior to any exposure to either methamphetamine (METH) or doxycycline (DOX)-induced Tat expression to establish a behavioral baseline. When the METH exposure regimen began, mice were treated with a low dose (1 mg/ml) and slowly increased to a higher dose (6 mg/ml). After the first four-day block of injections, injections began with a mid-range dose (3 mg/mL) and ended with the highest dose (6 mg/ml). Mice were treated four days in a row at four different time points (1000, 1200, 1400, 1600 h) separated by a three-day period without injections. The four-day block of injection occurred four separate times and the entire regimen lasted 25 days. Mice were further assessed on the BPM near the end of the METH regimen (day 23) and on Day 32 (2 days after the end of DOX injections and 7 days following METH exposure).

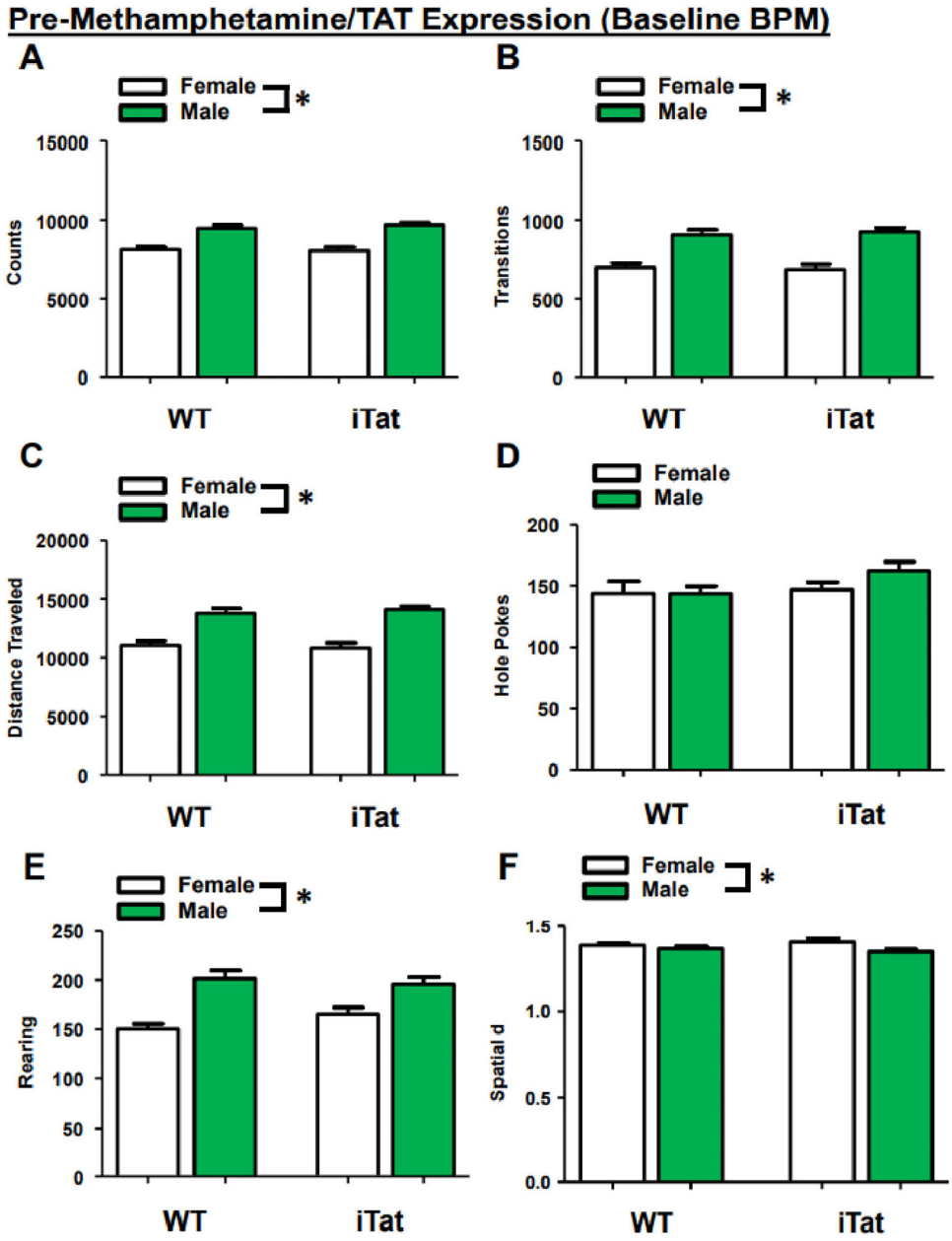

Fig. 2.

Sex determines baseline BPM activity, regardless of Tat promotor transgene expression. Compared to female mice, male mice exhibit higher total behavioral activity (*A*), transitions (*B*), and travel distance (*C*). Mice have similar holepoking behavior regardless of sex (*D*). While male mice exhibit higher rearing behavior (*F*), female mice have higher spatial d (*F*). Data presented as mean ± SEM. * = $p < 0.05$.

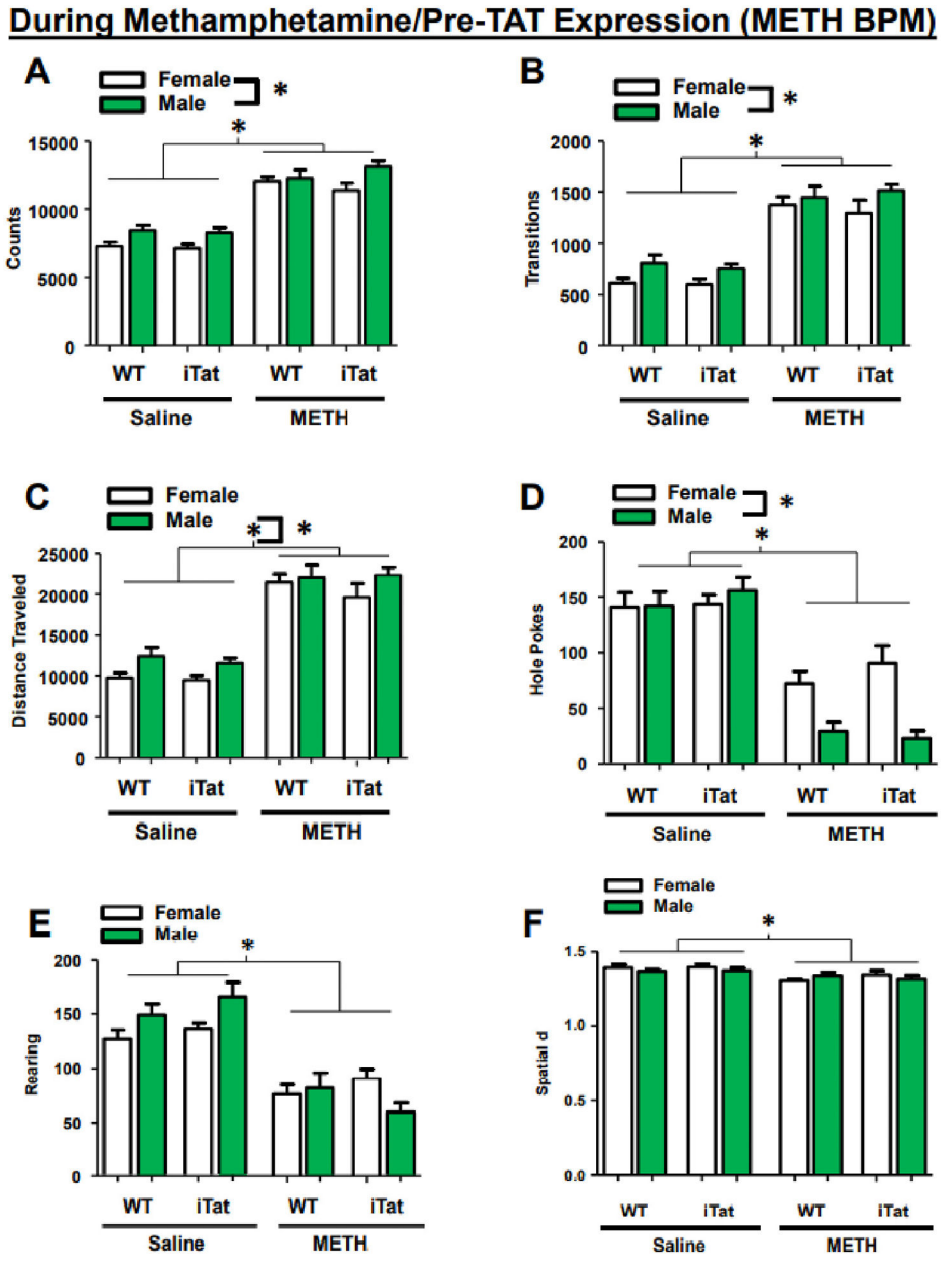

Fig. 3.

Previous exposure to METH alters activity in the BPM. Mice previously exposed to METH exhibited higher total behavioral activity (*A*), transitions (*B*), and travel distance (*C*), with all males showing higher scores compared to females across all groups. METH exposure also reduced holepoking behavior (*D*), rearing (*E*), and spatial d (*F*). Holepoking behavior showed a sex × drug interaction, with male mice performing significantly lower than female mice (*D*). Data presented as mean ± SEM. * = $p < 0.05$.

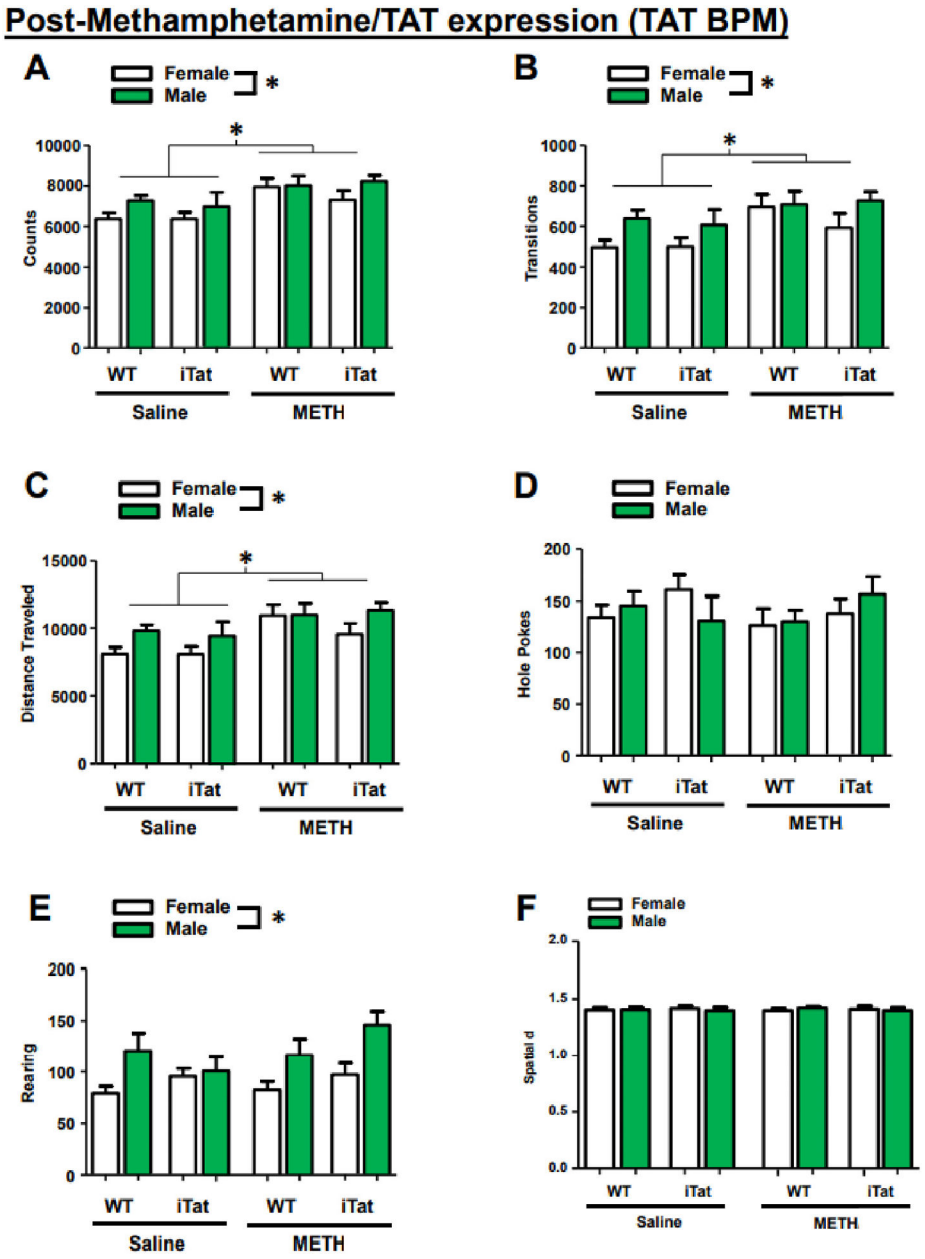

Fig. 4.

Movement in the BPM remains elevated long after METH exposure, but performance is unaltered by Tat expression. Five days after the end of the METH exposure regimen, METH-treated mice (and males across all groups) continue to display elevated total behavioral counts (*A*), transitions (*B*), and travel distance (*C*). METH treatment and Tat expression do not appear to affect holepokes (*D*), rearing (*E*), or spatial d (*F*). However, males across all groups exhibit higher rearing behavior than females (*E*). Data presented as mean ± SEM. * = $p < 0.05$.

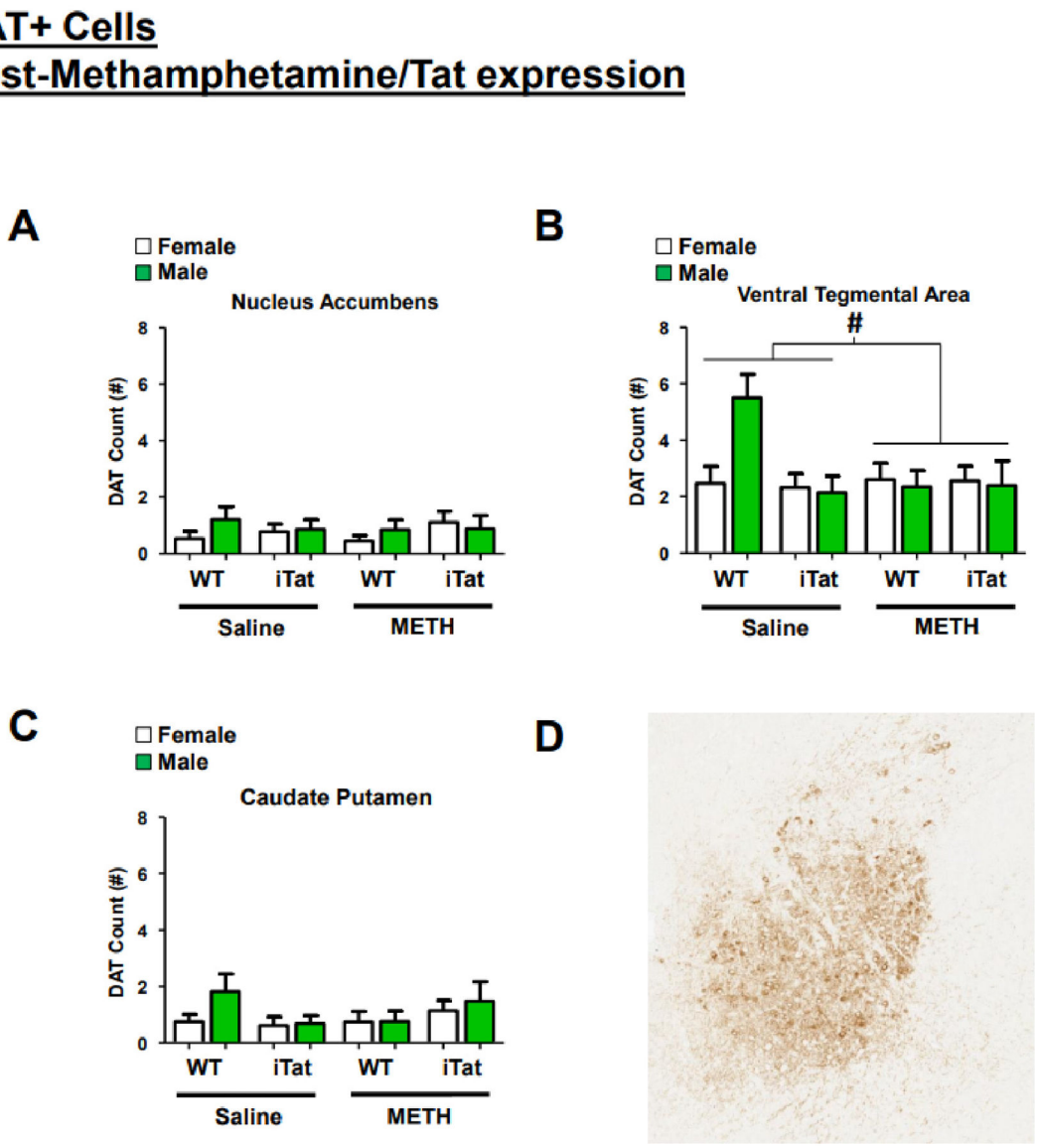

Fig. 5.

DAT expression is slightly altered in the ventral tegmental area. After the third and final BPM assessment, mice were sacrificed and brain were taken for immunostaining to determine the effects of METH exposure and Tat expression on the dopamine transporter (DAT). No effects were seen on DAT expression in the nucleus accumbens (*A*). In the ventral tegmental area, saline-treated mice tended to have higher DAT expression, driven mainly by WT, male mice (*B*). Neither sex, METH exposure, nor Tat expression altered DAT expression in the caudate putamen (*C*). A representative image of immunohistochemistry for DAT (brown) on a paraffin embedded mouse brain, sagittal section, ventral tegmental area. Original magnification 5×; no counterstaining (*D*). Data presented as mean ± SEM. # = $p <$ 0.1.