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Increases in compulsivity, inflammation, and neural injury in HIV transgenic rats with escalated methamphetamine self-administration under extended-access conditions

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Abstract

The abuse of stimulants, such as methamphetamine (METH), is associated with treatment non-compliance, a greater risk of viral transmission, and the more rapid clinical progression of immunological and central nervous system human immunodeficiency virus (HIV) disease. The behavioral effects of METH in the setting of HIV remain largely uncharacterized. We used a state-of-the-art paradigm of the escalation of voluntary intravenous drug self-administration in HIV transgenic (Tg) and wildtype rats. The rats were first allowed to self-administer METH under short-access (ShA) conditions, which is characterized by a nondependent and more “recreational” pattern of METH use, and then allowed to self-administer METH under long-access (LgA) conditions, which leads to compulsive (dependent) METH intake. HIV Tg and wildtype rats self-administered equal amounts of METH under ShA conditions. HIV Tg rats under LgA METH self-administration following a 4-week enforced abstinence period to model the intermittent pattern of stimulant abuse in humans, developed greater motivation to self-administer METH and self-administered larger amounts of METH. Impairments in function of the medial prefrontal cortex (mPFC) contribute to compulsive drug and alcohol intake. Gene expression profiling of the mPFC

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in HIV Tg rats with a history of escalated METH self-administration under LgA conditions showed transcriptional evidence of increased inflammation, greater neural injury and impaired aerobic glucose metabolism than wildtype rats that self-administered METH under LgA conditions. The detrimental effects of the interaction between neuroHIV and escalated METH intake on the mPFC are likely key factors in the greater vulnerability to excessive drug intake in the setting of HIV.

Introduction

Human immunodeficiency virus (HIV)-infected individuals have a higher risk of substance abuse, such as methamphetamine, opioids, and alcohol, than the general U.S. population (Cook et al., 2001; Dansak, 1997; Kumar, 2014; Meyerhoff, 2001; Petry, 1999; Samet et al., 2004; Silverstein and Kumar, 2014). Substance abuse is associated with treatment non-compliance, a greater risk of viral transmission, and more rapid clinical progression of HIV disease (Bing et al., 2001; Cadet and Krasnova, 2007; Chana et al., 2006; Galvan et al., 2002; Goodkin et al., 1998; Lucas et al., 2001; Lucas et al., 2002; Lucas et al., 2006; Nath, 2010; Nelson et al., 2002; Scott et al., 2007; Volkow et al., 2007). The abuse of stimulants, such as METH and cocaine, appears to adversely affect cognition in the setting of HIV (Carey et al., 2006; Levine et al., 2006; Nath et al., 2001; Nath, 2010; Scott et al., 2007; Soontornniyomkij et al., 2016). Methamphetamine use is also associated with higher depression scores in HIV-infected individuals (Blackstone et al., 2013; Bousman et al., 2009).

The behavioral effects of METH in the setting of HIV remain largely uncharacterized. The clinical literature suggests that HIV may itself contribute to the perpetuation of METH abuse. HIV patients report using METH to self-medicate HIV-related symptoms (e.g., depression and fatigue) and improve social-cognitive functioning (Gorman, 1996; Homer et al., 2008; Robinson and Rempel, 2006; Semple et al., 2002).

To explore the effects of METH abuse in the setting of HIV, we used voluntary intravenous METH self-administration in HIV transgenic (Tg) rats. Most research to date has focused on the effects of the HIV proteins gp120 and Tat, but evidence indicates that other HIV proteins (e.g., gp41, Nef, and Vpr, among others) also possess neurotoxic potential (Adamson et al., 1999; Jones et al., 2007; Patel et al., 2002; Trillo-Pazos et al., 2000; van de Bovenkamp et al., 2002). Thus, we utilized HIV Tg rats that express multiple HIV products in disease-relevant glial cells, such as microglia and astrocytes, but not in neurons (Reid et al., 2001; Repunte-Canonigo et al., 2014b; Royal et al., 2012).

HIV Tg rats exhibit changes in gene expression that are consistent with HAND in humans (Repunte-Canonigo et al., 2014b; Sanna et al., 2017). HIV Tg rats exhibit deficits in working memory (Repunte-Canonigo et al., 2014b), spatial learning (Vigorito et al., 2007), and performance in a reversal learning task (Lashomb et al., 2009). This is consistent with previous observations that pathological changes that are associated with neuroAIDS, such as synaptodendritic injury, can be induced by HIV proteins in the absence of viral infection and replication (Iskander et al., 2004; Kim et al., 2003; Toggas et al., 1994).

The occasional but limited use of a drug is clinically distinct from escalated drug use, which is characterized by an inability to limit drug intake, the emergence of chronic compulsive drug-seeking behavior, and vulnerability to relapse after cessation (Koob and Le Moal, 2005). Initial and occasional drug use is motivated by a positive emotional state (e.g., euphoria) that is induced by the drug, also described by the process of positive reinforcement (Koob and Le Moal, 2005). Self-administration studies of the neuropharmacological basis of the acute reinforcing effects of drugs of abuse (positive reinforcement) have traditionally used models of limited daily access to drug self-administration (Koob et al., 2004). Using these models, rats and mice with indwelling intravenous catheters are allowed to self-administer drugs of abuse for less than 3 h/day, producing stable levels and patterns of intake. However, addiction is characterized by the loss of control in limiting intake and compulsion to take the drug (Koob and Le Moal, 2005). Animal models of extended daily access to intravenous drug self-administration have been developed for cocaine, heroin, alcohol, nicotine, and METH, which result in escalated levels of intake (Ahmed and Koob, 1998; Cohen et al., 2012; George et al., 2007a; George et al., 2007b; George et al., 2014; Kitamura et al., 2006; Koob et al., 2004; Koob and Le Moal, 2005; Orio et al., 2010).

The present study used intravenous METH self-administration initially under short-access (ShA) conditions, which is characterized by a nondependent “recreational-like” pattern of METH use, followed by METH self-administration under long-access (LgA) conditions, which leads to compulsive (dependent) METH intake. Animals that self-administer METH under LgA conditions exhibit an upward shift of the METH dose-response function, which are indicative of compulsive drug self-administration and working memory impairments (Recinto et al., 2012)(Kitamura et al., 2006; Orio et al., 2010; Wee et al., 2007). Additionally, intracranial self-stimulation (ICSS) reward thresholds gradually become elevated in animals that self-administer METH under LgA conditions and are correlated with an increase in METH intake (Jang et al., 2013). The latter finding indicates that extended access to METH recruits negative reinforcement mechanisms, in which a depressive-like state of withdrawal drives compulsive responding for the drug to relieve the depressive-like state, which is not observed with recreational drug use (Jang et al., 2013; Koob and Le Moal, 2006; Koob and Le Moal, 2005; Koob and Le Moal, 2008; Koob and Volkow, 2010). The model of extended-access self-administration that is used in our laboratory and by others has been shown to recapitulate all seven of the diagnostic criteria for Substance Use Disorder in the *Diagnostic and Statistical Manual of Mental Disorders*, 4th edition (DSM-IV; American Psychiatric Association, 1994), and seven of the 11 criteria in the DSM-5 (George et al., 2014).

Impairments in frontostriatal connectivity are involved in both the progression to compulsive drug intake and neuroHIV (Feil et al., 2010; Ipser et al., 2015; Plessis et al., 2014; Volkow and Morales, 2015), suggesting that METH and HIV can additively promote compulsive drug intake and cognitive impairment. Thus, to better understand the molecular basis of detrimental interactions between HIV and METH, we profiled gene expression from the medial prefrontal cortex (mPFC) in HIV Tg and wildtype (WT) rats with a history of METH self-administration under LgA conditions. Previous studies showed that changes in gene expression that are associated with compulsive cocaine, heroin, and alcohol self-

administration are considerably different from changes in gene expression that are induced by a moderate “recreational-like” pattern of self-administration (Ahmed et al., 2005; Chen et al., 2013; Francesconi et al., 2009; Koob et al., 2004; Repunte-Canonigo et al., 2007; Repunte-Canonigo et al., 2010a; Repunte-Canonigo et al., 2010b; Repunte-Canonigo et al., 2014a; Repunte-Canonigo et al., 2015).

In the present study, HIV Tg rats that self-administered METH under LgA conditions exhibited greater drug seeking and taking than WT rats. The mPFC gene expression analysis showed that HIV Tg rats that self-administered METH under LgA conditions exhibited evidence of increases in neuroinflammation and neurodegeneration, a decrease in the tricarboxylic acid (TCA) cycle, and an increase in glycolysis gene expression.

Collectively, the present results indicate that the combination of neuroHIV and escalated METH intake results in significant additive neurotoxicity in the mPFC. The detrimental effect of neuroHIV and escalated METH intake on the expression of genes that are related to immune system activation and inflammation, neural damage, and metabolism may be significant contributors to neural injury and hypofrontality, which are believed to be key factors in compulsive drug taking and cognitive dysfunction in the HIV setting.

Results

Increase in METH self-administration in HIV transgenic rats

HIV Tg rats were first trained to self-administer METH intravenously under ShA conditions and then under LgA conditions. HIV Tg rats exhibited similar patterns of acquisition and intake under ShA conditions but then greater compulsivity after the escalation of METH intake under LgA conditions. During the pre-escalation phase under ShA conditions (1 h/day access to METH self-administration), mixed factorial analysis of variance (ANOVA), with group as the between-subjects factor and session as the within-subjects factor, indicated no differences in METH intake between HIV Tg and littermate control WT rats during the pre-escalation phase ($F_{11,275} = 3.39$, $p > 0.05$; Fig. 1A).

The rats were then switched to LgA conditions (6 h/day access to METH self-administration), which induced the escalation of METH intake ($p < 0.01$, day 5 *vs.* day 1 in the WT group; $p < 0.01$, day 10 *vs.* day 1 in the HIV Tg group; separate one-way ANOVAs followed by Fisher’s Least Significant Difference [LSD] *post hoc* test). Mixed factorial ANOVA, with group as the between-subjects factor and session as the within-subjects factor, showed a significant group × session interaction ($F_{15,330} = 8.48$, $p < 0.001$). Fisher’s LSD *post hoc* test showed significant differences in the number of METH infusions between WT and HIV Tg rats between sessions 5 and 8 ($p < 0.01$).

After re-escalation following a 4-week enforced abstinence period, during which METH was unavailable to model the intermittent pattern of stimulant abuse in humans, mixed factorial ANOVA, with group as the between-subjects factor and session as the within-subjects factor, showed a significant group × session interaction ($F_{7,147} = 3.263$, $p < 0.01$. Fisher’s LSD *post hoc* test showed a significant increase in METH intake in HIV Tg rats *vs.* WT rats starting from session 5 ($p < 0.01$). A progressive-ratio (PR) test was conducted to measure the

motivation for METH intake following the escalation of METH self-administration (before re-escalation). HIV Tg rats exhibited an increase in compulsive-like responding for METH under the PR schedule of reinforcement ($t_{22} = 2.402$, $p < 0.05$).

Overall, HIV Tg rats developed greater compulsivity following the escalation of METH intake under LgA conditions after re-exposure to METH self-administration following a 4-week enforced abstinence period, reflected by greater responding under both fixed-ratio (FR) and PR schedules of reinforcement. These results indicate that HIV interacts with the effects of a history of binging-abstinence patterns of METH intake to increase vulnerability to relapse and motivation for the drug.

Gene expression profiling in the mPFC in HIV Tg and WT rats that self-administered METH under LgA conditions

Functional impairments in the mPFC contribute to compulsive drug intake. Therefore, we profiled gene expression in the mPFC in HIV Tg rats with a history of escalated METH self-administration (Fig. 2). Among the differentially expressed genes were F-Box and WD repeat domain containing 11 (FBXW11; also known as β-transducin repeat containing protein 2 [βTrCP2], homolog of Slimb [HOS]), interferon α (IFNα) inducible protein 27 (IFI27; (Pulliam, 2014), ubiquitin domain containing 2 (UBTD2; (Song et al., 2010), and potassium voltage-gated channel interacting protein 1 (KCNIP1 or KChIPI; (An et al., 2000); Fig. 2A, B). The expression of FBXW11 significantly decreased in HIV Tg rats with a history of escalated METH self-administration (Fig. 2A, B). FBXW11 is an E3 ubiquitin ligase that is implicated in downregulating the interferon type I receptor (IFNAR1) in response to the induction of IFNα (Kumar et al., 2003) and involved in the transcriptional activation of nuclear factor-κB (NF-κB) by promoting the degradation of the inhibitor of κB (IκB; (Suzuki et al., 2000). The expression of IFI27 significantly increased in HIV Tg rats with a history of escalated METH self-administration (Fig. 2A, B). IFI27 is a gene that mediates antiviral activity in response to neurotropic viruses (Cho et al., 2013) and is a proposed biomarker of monocyte activation (Pulliam, 2014). UBTD2 is a ubiquitin-like protein with an unknown function that can bind ubiquitin, indicating that it may play a role in regulating the ubiquitination of protein substrates (Song et al., 2010). The expression of KCNIP1 significantly increased in HIV Tg rats with a history of escalated METH self-administration (Fig. 2A, B). KCNIP1 is a member of a family of small cytosolic, calciumbinding proteins that were initially identified as subunits of K_v4 potassium channels, which regulate the gating of voltage-gated A-type potassium currents in neuronal tissue and the heart (An et al., 2000). The association between KCNIP1 and K_v4 results in an increase in current, the acceleration of recovery from inactivation, slower inactivation kinetics, and lower neuronal excitability (Gonzalez et al., 2014). KCNIP1 was shown to promote the stronger inhibitory control of firing during sustained activity (Bourdeau et al., 2011). Top differentially expressed genes in HIV Tg rats that self-administered METH under LgA conditions are shown in Fig. 2C. Top differentially expressed pathways are shown in Fig. 2D. Gene expression differences between HIV Tg and WT rats are in part preexisting, consistent with earlier observations (Repunte-Canonigo et al. 2014) and partially attributable to the interaction between HIV and a history of METH intake and re-escalation.

Transcriptional evidence of an increase in neuroinflammation in HIV Tg rats that self-administered METH under LgA conditions

HIV Tg and WT rats that self-administered METH under LgA conditions exhibited a prominent differential expression of pathways related to immune activation and inflammation. These include pathways that are indicative of general immune activation, inflammation, and greater cytokine signaling (Fig. 3A–D). Among the activated pathways were pathways that are related to tumor necrosis factor α (TNF-α) and inflammasome activation (Fig. 3E–G) and the recruitment of specific cytokine signaling pathways, including interleukin-1β (IL-1β, a pivotal proinflammatory cytokine that is released following inflammasome activation; Fig. 3D), IL-2, and IL-3 signaling (Fig. 3H, I). IL-3 is associated with active inflammatory processes in the brain (Renner et al., 2016) and has been suggested to induce a neuroprotective phenotype in microglia (Choudhury et al., 2011). Lastly, HIV Tg and WT rats that self-administered METH under LgA conditions exhibited a more pronounced induction of both type I and II IFN signaling (Fig. 3J–L)

Transcriptional evidence of increase in neural injury in HIV Tg rats that self-administered METH under LgA conditions

Differences in the regulation of pathways that are involved in neurodegeneration between HIV Tg and WT rats that self-administered METH under LgA conditions suggest the differential activation of pathogenic mechanisms (Fig. 4). These mechanisms involved the greater induction of pathways that are implicated in neuronal damage, such as genes related to complement (Fig. 4A), amyotrophic lateral sclerosis (Fig. 4B), and the activation of glucocorticoid signaling (Fig. 4C). Escalated METH self-administration interacted with HIV to affect the expression of genes that are involved in the extracellular matrix (Fig. 4D–F) and differential expression of genes related to DNA damage and repair (Fig. 4G–I).

Differences in the regulation of pathways that are involved in metabolism between HIV Tg and WT rats that self-administered METH under LgA conditions were also observed (Fig. 5). Pathway analysis indicated a decrease in genes related to glucose oxidation through the tricarboxylic acid (TCA) cycle and an increase in glycolysis in HIV Tg rats that self-administered METH under LgA conditions compared with METH-self-administering WT rats (Fig. 5).

Pathway analysis also indicated a greater catabolic molecular environment in HIV Tg rats that self-administered METH under LgA conditions (Fig. 6). The gene expression analysis in METH-self-administering HIV Tg rats suggested lower protein synthesis, higher protein degradation (Fig. 4A–C), and impairments in RNA synthesis and processing (Fig. 6D–F).

Discussion

The present study used paradigms of voluntary intravenous METH self-administration under ShA conditions (characterized by a “recreational” pattern of use) followed by LgA conditions (which lead to compulsive/dependent intake; (Kitamura et al., 2006; Orio et al., 2010; Recinto et al., 2012; Wee et al., 2007) in HIV Tg and WT rats. HIV Tg and WT rats self-administered equal amounts of METH under ShA conditions. However, HIV Tg rats

self-administered greater amounts of METH and exhibited greater motivation for METH than WT rats under LgA METH self-administration following a 4-week enforced abstinence period to model the intermittent pattern of stimulant abuse in humans.

HIV Tg rats are a small-animal model of concomitantly low levels of expression of multiple HIV products in disease-relevant cells in the central nervous system, such as microglia and astrocytes, but not in neurons (Reid et al., 2001; Repunte-Canonigo et al., 2014b; Royal et al., 2012), which is a key characteristic of HAND in the setting of viral suppression. HIV Tg rats differ from most of the currently available animal models of HIV that usually focus on a single viral protein. HIV Tg rats exhibit changes in gene expression that are consistent with human neuroHIV (Repunte-Canonigo et al., 2014b; Sanna et al., 2017).

The present model of the escalation of intravenous METH self-administration has not been previously utilized in the HIV/AIDS field. This is an innovative paradigm that was developed at The Scripps Research Institute, which effectively models the negative reinforcement that is associated with escalated drug intake but is not observed with recreational drug use (Ahmed and Koob, 1998; Jang et al., 2013; Koob and Le Moal, 2006; Koob and Le Moal, 2005; Koob and Le Moal, 2008; Koob and Volkow, 2010) and is associated with cognitive impairment and a depressive-like state during withdrawal (Ahmed et al., 2002; Jang et al., 2013; Koob and Kreek, 2007; Koob, 2009; Koob and Volkow, 2010; Recinto et al., 2012). Negative reinforcement is characterized by a depressive-like state during abstinence that drives compulsive drug seeking to relieve the negative emotional state (Ahmed et al., 2002; Jang et al., 2013; Koob and Le Moal, 2006; Koob and Le Moal, 2005; Koob and Le Moal, 2008; Koob and Volkow, 2010). In this paradigm, cognitive deficits that involve impairments in mPFC function emerge during the transition to escalated drug intake and contribute to compulsive drug seeking and taking (Jang et al., 2013; Recinto et al., 2012). Hypofrontality characterizes both compulsive drug intake in dependent individuals and cognitive dysfunction in neuroHIV (Feil et al., 2010; Goldstein and Volkow, 2002; Goldstein and Volkow, 2011; Ipser et al., 2015; Plessis et al., 2014; Volkow et al., 1992a; Volkow et al., 1992b; Volkow and Morales, 2015; Volkow et al., 2015; Wiers et al., 2016), suggesting a neurobiological basis for the additive effects of METH and HIV on compulsive drug intake, as seen in the present study, as well as cognitive impairment.

The identification of factors that promote relapse or resilience is of particular interest (Goldstein and Volkow, 2011). HIV Tg rats in the present study developed greater compulsivity to self-administer METH following the re-escalation (post-abstinence relapse) of METH intake after a 4-week enforced abstinence period, despite acquiring escalated METH self-administration behavior later than their WT littermates. When access to METH self-administration was restored after the 4-week respite, HIV Tg rats exhibited greater METH intake on both FR 8and PR schedules of reinforcement. Binging patterns of stimulant abuse are prevalent in certain segments of people living with HIV (Harzke et al., 2009; Roy et al., 2017; Wechsberg et al., 2003). Studies in both rats and humans indicate that the motivational impact of drug-associated cues on behavior becomes progressively stronger over periods of repeated abstinence, referred to as the “incubation of craving,” which increases the vulnerability to relapse and represents a challenge to successful treatment (Grimm et al., 2001; Parvaz et al., 2016). Increases in the incubation of cue-

induced craving were found to predict the rate and extent of the re-escalation of cocaine intake after a 4-week period of enforced abstinence in rats (Guillem and Ahmed, 2018). In the present study, HIV Tg and WT littermate controls did not differ in their levels of drug intake before the period of enforced abstinence. Thus, the present results indicate that HIV interacts with the effects of binging-abstinence patterns of METH intake to increase the vulnerability to relapse and motivation for the drug.

To investigate the molecular mechanisms that underlie interactions between HIV and METH, we performed gene expression profiling of the mPFC, which has been consistently implicated in craving and the escalation of drug taking (Du et al., 2018; George et al., 2008; Goldstein and Volkow, 2011). Here, we show that the increase in METH self-administration in HIV Tg rats under LgA conditions was associated with an increase in inflammation. We found a global and pronounced increase in the activation of immune and inflammation-related pathways, including pathways that are related to cytokine and both type I and type II IFN signaling, which have been implicated in neural cell injury and cognitive impairment in HIV (Pulliam, 2014).

Inflammation has been associated with the aging brain and degenerative diseases (Mejias et al., 2018; Youm et al., 2013). We observed gene expression evidence of greater neural injury and neurodegeneration in HIV Tg rats *vs.* WT rats that self-administered METH under LgA conditions. This included pathways that are involved in aging and neurodegenerative diseases (e.g., Alzheimer's disease), including glucocorticoid receptor-regulated genes (Djamshidian and Lees, 2014; Hartmann et al., 1997; Hou et al., 2014; Notarianni, 2013; Vyas and Maatouk, 2013), and pathways that are involved in extracellular matrix deposition (Zhang et al., 2014). Previous studies showed that stimulants, such as cocaine and METH, affect extracellular matrix heparan sulfate content and sulfation levels (Chen et al., 2013; Chen et al., 2017). Heparan sulfate has also been implicated in amyloid-β peptide and tau fibrillization in Alzheimer's disease (Liu et al., 2016).

We observed a reduction of the expression of TCA cycle-related genes, with an increase in glycolysis-related genes in HIV Tg rats with a history of METH self-administration under LgA conditions compared with WT rats that self-administered METH under the same conditions. A decrease in oxidative phosphorylation reflects mitochondrial impairment that leads to less efficient energy metabolism. HIV has been shown to variably affect the TCA cycle in different cell types. The downregulation of TCA proteins has been observed in major blood types of HIV non-progressors (Zhang et al., 2017). The decrease in oxidative phosphorylation may reflect a hypometabolic state that is associated with neurodegenerative alterations in neurons (Butterfield and Halliwell, 2019). Higher lactate levels were seen in the brain in HIV patients in the pre-cART era, attributed to greater anaerobic glycolytic demands of macrophages associated with inflammation (Barker et al., 1995; Simone et al., 1998).

We also observed an apparent increase in the catabolic milieu that was elicited by the interaction between METH and HIV. We observed a reduction of the transcription of genes that are related to RNA processing and a reduction of genes that are related to translation. The dysregulation of translation is emerging as a possible mechanism of

neurodevelopmental disease and neurodegeneration (Darnell and Klann, 2013; Halliday et al., 2017; Hetman and Slomnicki, 2019).

In conclusion, HIV Tg rats self-administered more METH than WT rats under extended-access conditions, leading to an increase in the escalation of METH intake following an intermittent pattern of access to the drug. Dysfunction of the mPFC contributes to compulsivity in the present paradigm. Prefrontal cortex gene expression analyses indicated that HIV Tg rats that escalated their METH intake exhibited transcriptional evidence of increases in neuroinflammation and neurodegeneration and alterations of energy metabolism. These alterations may be key contributors to hypofrontality that contributes to the vulnerability to excessive drug intake in the setting of dependence.

Materials and Methods

Animals

Male HIV Tg rats (n = 13) and littermate control WT rats (n = 14) on a mixed Fischer/Wistar background (originally on a Fischer background, crossed for three generations with Wistar rats) were housed two per cage on a reverse 12 h/12 h light/dark cycle (lights off at 8:00 AM) in a temperature (20-22°C) and humidity (45-55%) controlled vivarium with *ad libitum* access to tap water and food pellets (PJ Noyes, Lancaster, NH, USA). All of the procedures were conducted in strict adherence to the National Institutes of Health *Guide for the Care and Use of Laboratory Animals* and were approved by the Institutional Animal Care and Use Committee of The Scripps Research Institute. At the time of testing, the rats' body weights ranged between 350 and 400 g.

Intravenous catheterization

The animals were anesthetized by the inhalation of a mixture of isoflurane, and intravenous catheters were aseptically inserted in the right jugular vein using a modified version of a procedure that was described previously (Caine and Koob, 1993; de Guglielmo et al., 2013). The vein was punctured with a 22-gauge needle, and the tubing was inserted and secured inside the vein by tying the vein with suture thread. The catheter assembly consisted of an 18 cm length of Micro-Renathane tubing (0.023 inch inner diameter, 0.037 inch outer diameter; Braintree Scientific, Braintree, MA, USA) that was attached to a guide cannula (Plastics One, Roanoke, VA, USA). The guide cannula was bent at a near right angle, embedded in dental acrylic, and anchored with 2 cm square mesh. The catheter exited through a small incision on the back, and the base was sealed with a small plastic cap and metal cover cap. This design helped keep the catheter base sterile and protected. The catheters were flushed daily with heparinized saline (10 U/ml of heparin sodium; American Pharmaceutical Partners, Schaumburg, IL, USA) in 0.9% bacteriostatic sodium chloride (Hospira, Lake Forest, IL, USA) that contained 20 mg/0.2 ml of the antibiotic Timentin (GlaxoSmithKline, UK).

Drugs

Methamphetamine HCl (National Institute on Drug Abuse, Bethesda, MD, USA) was dissolved in 0.9% saline (Hospira, Lake Forest, IL, USA) and self-administered intravenously at a dose of 0.05 mg/kg/infusion.

Operant training

Self-administration was performed in operant conditioning chambers (Med Associates, St. Albans, VT, USA) that were enclosed in lit, sound-attenuating, ventilated environmental cubicles. The front door and back wall of the chambers were constructed of transparent plastic, and the other walls were opaque metal. Each chamber was equipped with two retractable levers that were located on the front panel. Methamphetamine was delivered through plastic catheter tubing that was connected to an infusion pump. The infusion pump was activated by responses on the right (active) lever. Responses on the left (inactive) lever were recorded but did not have any scheduled consequences. Activation of the pump resulted in the delivery of 0.1 ml of the drug solution. A computer controlled fluid delivery and behavioral data recording.

All of the rats were tested for METH self-administration on a continuous reinforcement (FR1) schedule in three successive phases: pre-escalation, escalation, and re-escalation. Each active lever press resulted in the delivery of one METH dose (0.5 mg/kg/0.1 ml infusion). A 20-s timeout (TO) period followed each METH infusion. During the timeout period, responses on the active lever did not have scheduled consequences. This TO period occurred concurrently with the illumination of a cue light that was located above the active lever to signal delivery of the positive reinforcement.

At the end of the escalation phase, the rats were also tested on a PR schedule of reinforcement. Under these conditions, the response requirements that were necessary to receive a single drug dose increased according to the following equation: $(5e^{[\text{injection numbers} \times 0.2]}) - 5$. This resulted in the following progression of response requirements: 1, 2, 4, 6, 9, 12, 15, 20, 25, 32, 40, 50, 62, 77, 95, 118, 145, 178, 219, 268, etc. The breakpoint was defined as the last ratio that was attained by the rat prior to a 60 min period during which a ratio was not completed.

Pre-escalation phase: ShA.—During this phase, the rats were trained to acquire intravenous METH self-administration. All of the animals were allowed access to an FR1 TO 20 s schedule of METH self-administration with 1 h access to METH for 12 consecutive sessions (0.5 mg/kg/0.1 ml infusion).

Escalation phase. LgA.—This phase began 2 days after the end of the pre-escalation phase. During the escalation phase, drug access was increased to 6 h per LgA session. This phase lasted for 15 consecutive sessions and was followed by a PR test.

Re-escalation phase.—After the first escalation, the animals had no access to METH for 4 weeks. Following these 4 weeks of abstinence, the animals were allowed to self-administer METH in 6 h sessions according to the re-escalation protocol (8 sessions).

Total RNA isolation and RNA sequencing

Microdissected tissue from the mPFC was processed for total RNA isolation using the miRNeasy kit (Qiagen) and Zymo purification kit (Zymo Research). Libraries were prepared with the KAPA mRNA HyperPrep Kit for Illumina sequencing (KAPABiosystems) for mRNA capture with magnetic oligo-dT beads, cDNA synthesis, and library construction and amplification. The Poly-A libraries were subsequently sequenced on an Illumina HiSeq4000 sequencer at 30-million-read target coverage (100 bp paired-end reads).

Gene expression profiling and gene set enrichment analysis

The sequences were first trimmed using Trimmomatic with default setting (version 0.33). All of the samples passed the quality control by fastQC (version 0.11.3). Fastq files were aligned to combined rat (Rnor_6.0) and HIV-1 (NC_001802) transcriptomes on 8/8/2017 using Bowtie2 (Langmead et al., 2009) with default settings. Transcript expression was normalized using RSEM (version 1.3.0; (Li and Dewey, 2011). The two transcriptomes were combined as described previously (Fu et al., 2019). The rat genome was humanized using the biomaRt package from R. Only genes with the highest counts were kept when multiple rat genes matched to one human gene. Differential expression analysis was performed using the DESeq2 package in R (the Wald method was used). Gene set enrichment analysis (GSEA; (Subramanian et al., 2005) was performed in R for MSigDB curated gene sets but excluding perturbation-based gene sets for a total of 1,452 well-known MSigDB gene sets. Multiple testing adjustment was performed using the False Discovery Rate. Fastq files were deposited in the European Nucleotide Archive project PRJEB33332.

Quantitative PCR validation

Differentially expressed genes in the mPFC were validated in HIV and WT rats with or without a history of METH exposure using the SYBR Green fluorescence detection kit on an iQ5 instrument (Bio-Rad). A set of optimized real-time polymerase chain reaction (PCR) primer assays was designed, and the following sequences were used for the following targets: FBXW11 (AGCCCTGCTTCTGATTTACG [sense] and GAAGACTCTGGGTAGTCGTTTG [antisense]), UBTD2 (GGACTATGTGGTGCAGGTTAT [sense] and TAACAATAGAGCGGAGCCAAG [antisense]), KCNIP1 (ACGAATTTCTCGAGTCCTGTC [sense] and GCCAGTGTCCTCAGTTACAT [antiSense]), IFI27 (CAGCCAAGCTGATGTCTGT [sense] and AGGACCTTAGATGTCATGGAGA [antisense]), and β-actin (AGATTACTGCCCTGGCTCCT [sense] and CAGTGAGGCCAGGATAGAGC [antisense]). Gene expression was normalized to β-actin and analyzed based on the $^{\Delta\Delta}$CT method.

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- The abuse of stimulants, such as methamphetamine (METH), is associated with treatment non-compliance, a greater risk of viral transmission, and more rapid clinical progression of immunological and central nervous system (CNS) human immunodeficiency virus (HIV) disease.
- We used a state-of-the-art paradigm of escalated voluntary intravenous drug self-administration in HIV transgenic (Tg) and wildtype (WT) rats. The rats were first allowed to self-administer METH under short-access (ShA) conditions, which is characterized by a nondependent and more “recreational” pattern of METH use, and then under long-access (LgA) conditions, which leads to compulsive (dependent) METH intake.
- Compulsivity in METH abuse appears to be driven by medial prefrontal cortex (mPFC) dysfunction. Gene expression profiling of the mPFC in HIV Tg rats with a history of escalated METH self-administration under LgA conditions showed evidence of greater inflammation than WT rats that self-administered METH under LgA conditions, greater neural injury, lower aerobic and higher anaerobic glucose metabolism, and impairments in protein synthesis, a potential mechanism of neurodegeneration.
- These alterations may be key contributors to neural injury and hypofrontality that are believed to underlie cognitive dysfunction in HIV and greater vulnerability to excessive drug intake in the setting of dependence.

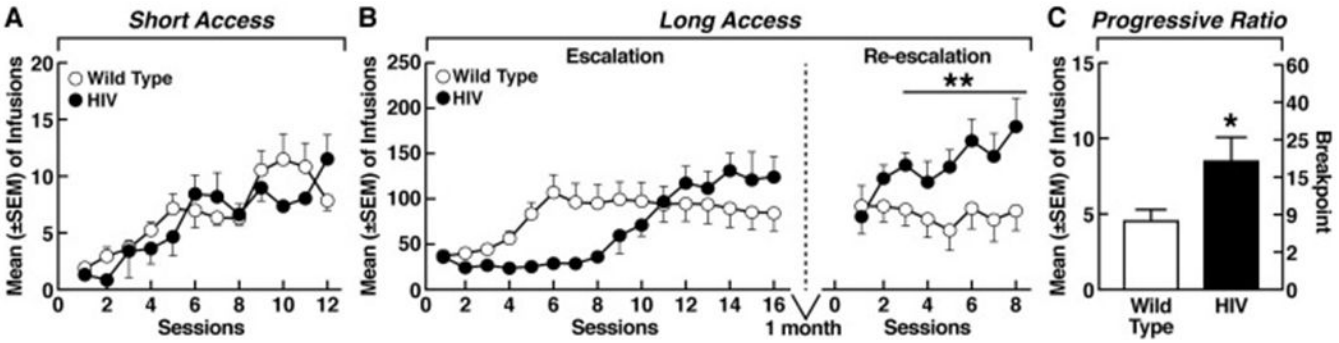

Fig. 1. HIV Tg rats exhibited greater compulsivity than WT rats for METH self-administration under long-access conditions.

(**A**) HIV Tg and littermate control wildtype (WT) rats were trained to self-administer methamphetamine (METH) under short-access conditions (ShA) (1 h/day) on a fixed-ratio 1 schedule of reinforcement. (**B**) The rats were then switched to long-access conditions (LgA) (6 h/day), resulting in the escalation of METH intake ($^{\#\#}p < 0.01$, day 5 *vs.* day 1 in the WT group; $^{\$\$}p < 0.01$, day 10 *vs.* day 1 in the HIV Tg group). Significant differences were detected in the number of METH infusions between WT and HIV Tg rats between sessions 5 and 8 ($^{++}p < 0.01$). After re-escalation, a significant increase in METH intake was detected in HIV Tg rats *vs.* WT rats starting from session 5 ($^{**}p < 0.01$). (**C**) Following the escalation of METH self-administration (before re-escalation), the rats were tested under a progressive-ratio schedule ($^{*}p < 0.05$, *vs.* WT). HIV Tg rats that self-administered METH under LgA conditions exhibited an increase in responding, reflected by increases in the number of infusions and breakpoints on the PR schedule of reinforcement.

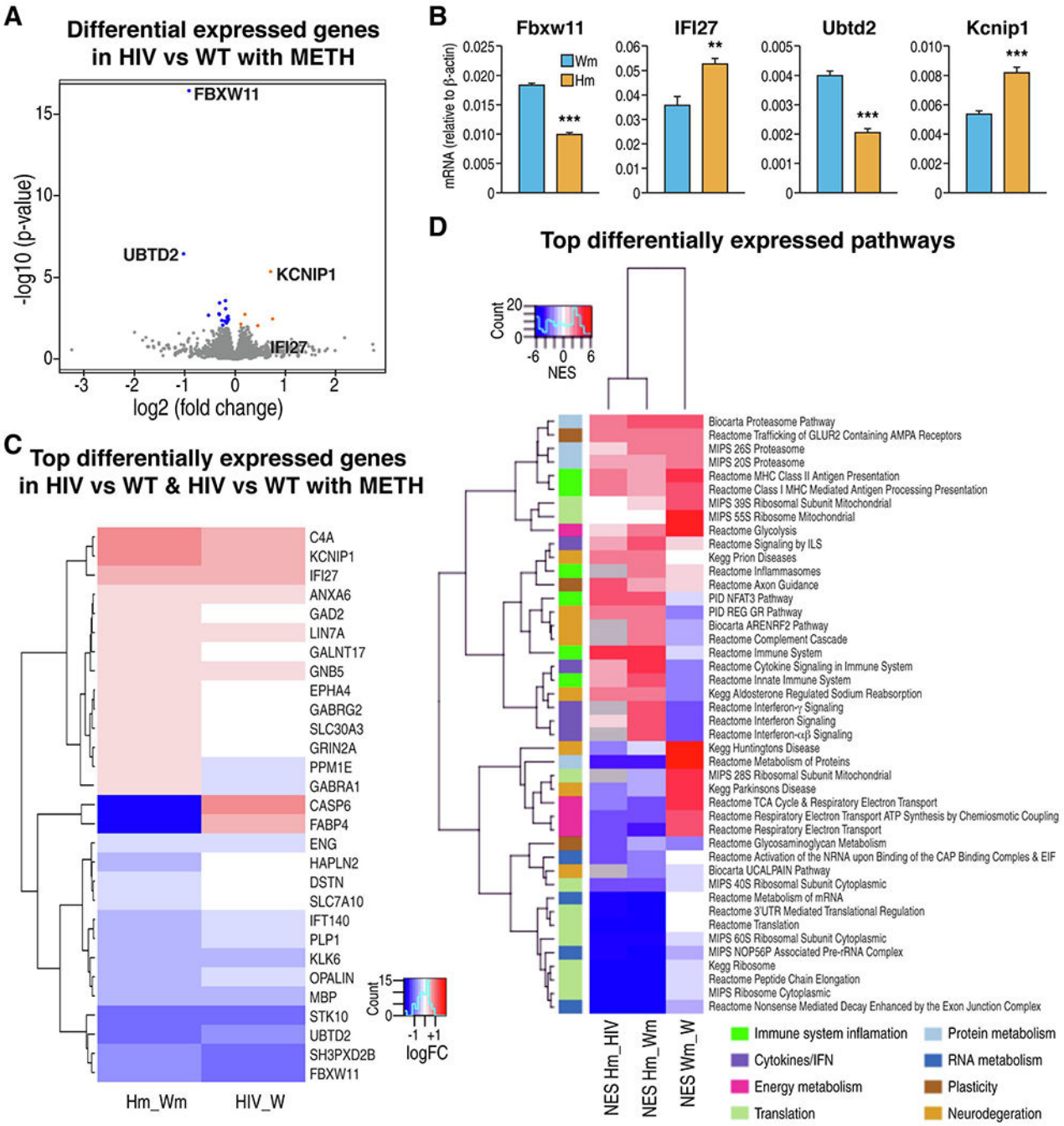

Fig. 2. Differential gene expression in HIV Tg rats and WT rats that self-administered METH under long-access conditions.

(**A**) Volcano plot of changes in gene expression in the medial prefrontal cortex (mPFC) in HIV Tg rats that self-administered METH under LgA conditions compared with METH-self-administering WT rats. The plots show significance (Log10 of *p* value) *vs.* fold-change (Log2) on the y and x axes, respectively. (**B**) RT-PCR validation of representative differentially expressed genes indicated in (A), including F-Box and WD repeat domain containing 11 (FBXW11), IFNα inducible protein 27 (IFI27), ubiquitin domain containing 2

(UBTD2), and potassium voltage-gated channel interacting protein 1 (KCNIP1). $^{**}p < 0.01$, $^{***}p < 0.0001$. Hm, HIV Tg rats that self-administered METH under LgA conditions; Wm, WT rats that self-administered METH under the same conditions. (**C**) Heatmap that depicts regulation of the top differentially expressed genes in HIV *vs.* WT (HIV_WT) and HIV *vs.* WT self-administering METH (Hm_Wm). (**D**) Heatmap of the top differentially expressed pathways indicated by the Gene Set Enrichment Analysis (GSEA) algorithm (Subramanian et al., 2005). NES, normalized enrichment score (Subramanian et al., 2005); Hm_HIV = HIV Tg rats that self-administered METH *vs.* HIV Tg rats; Hm_Wm = HIV Tg *vs.* WT rats that self-administered METH; Wm_W = WT rats that self-administered METH *vs.* WT rats. As further expanded in the next figures, differentially regulated pathways included pathways that are involved in inflammation and immune activation, energy metabolism, protein and RNA metabolism, and neurodegeneration.

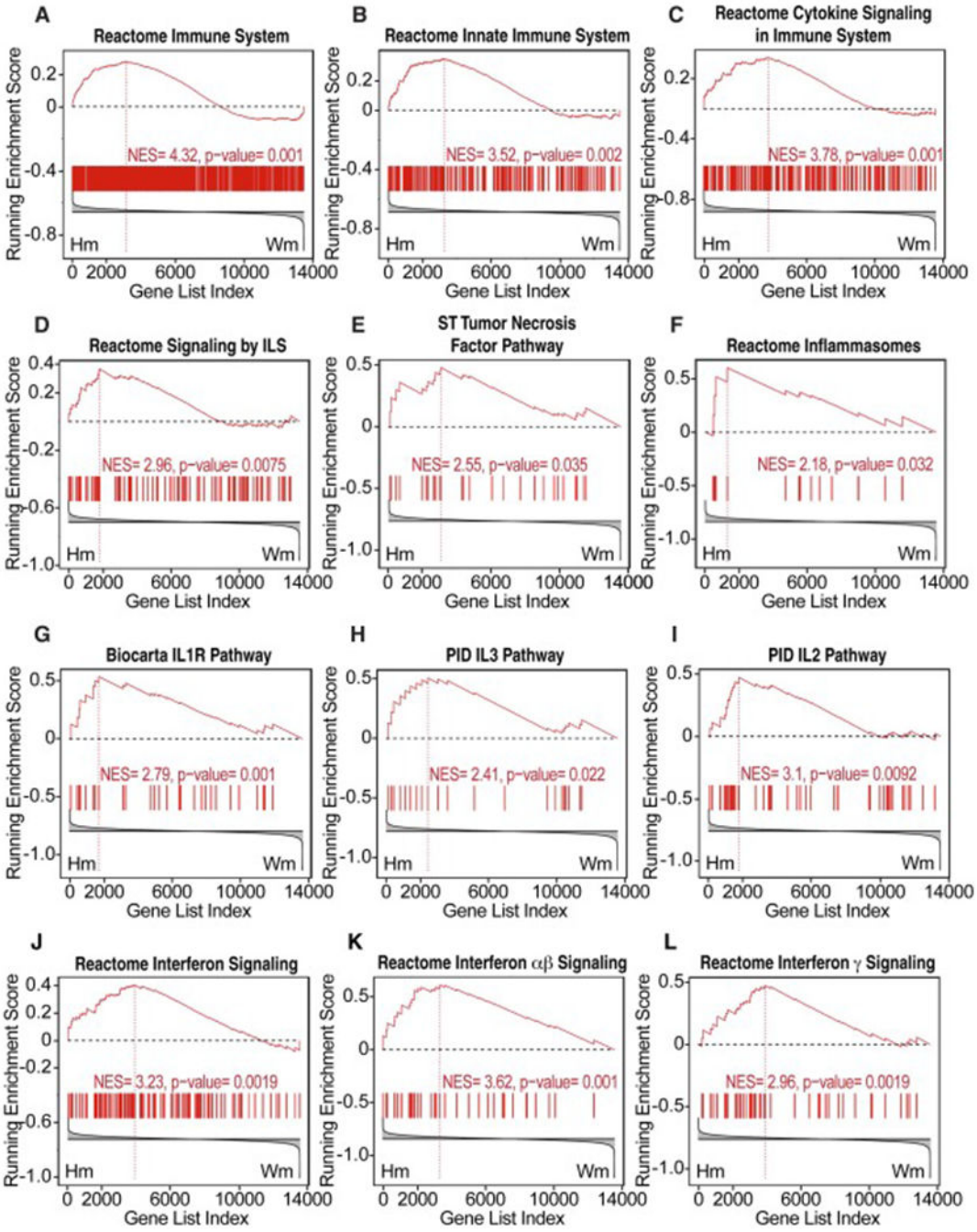

Fig. 3. Transcriptional evidence of an increase in neuroinflammation in HIV Tg rats that self-administered METH under long-access conditions.

Representative pathways are indicative of (**A, B**) broad immune activation and inflammation and (**C, D**) cytokine signaling, including (**E**) tumor necrosis factor α (TNF-α), (**F**) inflammasome activation, (**G-I**) IL-1, IL-2, and IL-3 activation, and (**J-L**) type I and II interferon (IFN) signaling, in the mPFC in HIV Tg rats that self-administered METH under LgA conditions (Hm) compared with METH-self-administering WT rats (Wm).

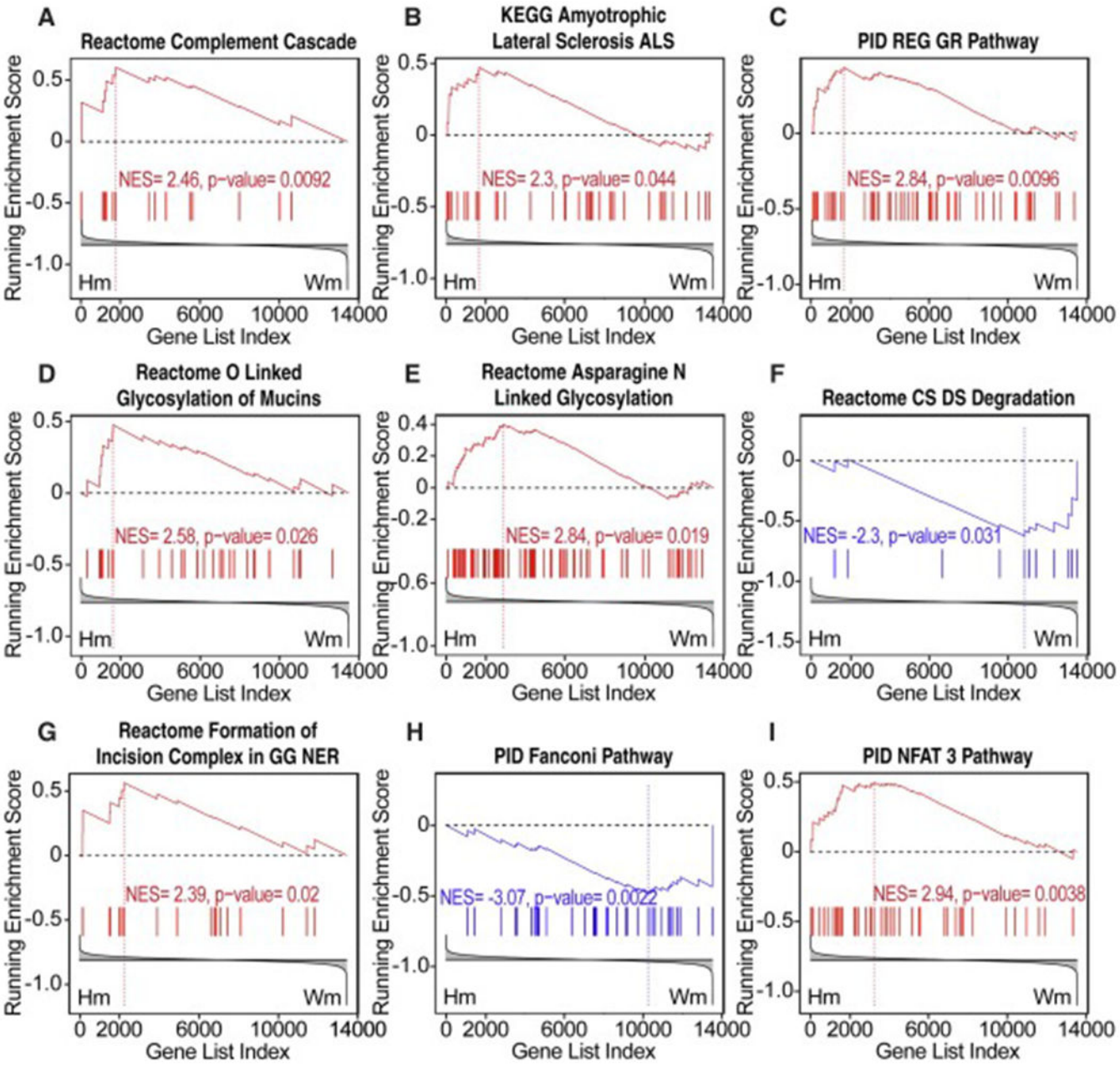

Fig. 4. Gene expression changes indicative of increase in neural damage in HIV Tg rats that self-administered METH under long-access conditions.

In the mPFC of HIV Tg rats that self-administered METH under LgA conditions (Hm) compared with METH-self-administering WT rats (Wm), increases were observed in the transcription of (**A**) complement-related genes; (**B**) genes that are related to neurodegeneration in amyotrophic lateral sclerosis; (**C**) genes that are related to glucocorticoid signaling; increases were also seen in the differential expression of pathways that are involved in (**D-F**) the extracellular matrix and (**G-I**) DNA damage and repair.

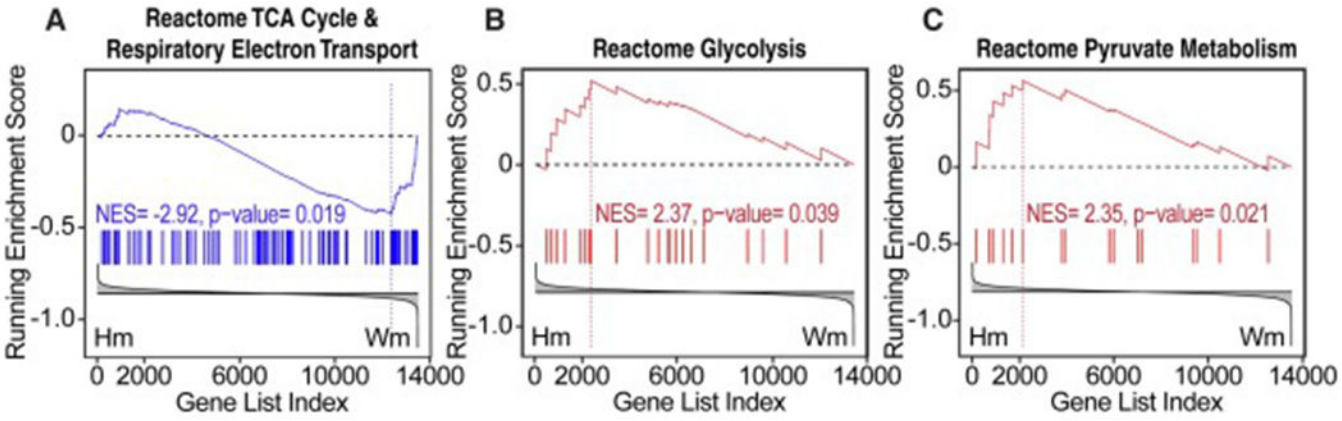

Fig. 5. Differential regulation of pathways that are involved in glucose metabolism between HIV Tg rats and WT rats that self-administered METH under long-access conditions.

(**A-C**) Decrease in the expression of genes that are related to glucose oxidation through the tricarboxylic acid (TCA) cycle (**A**) and increase in the expression of genes that are related to glycolysis (**B**) and the metabolism of pyruvate (**C**), the end product of glycolysis, in the mPFC in HIV Tg rats that self-administered METH under LgA conditions (Hm) compared with METH-self-administering WT rats (Wm). A reduction of oxidative phosphorylation through the TCA cycle may reflect a hypometabolic state that can contribute to neural damage and mPFC dysfunction.

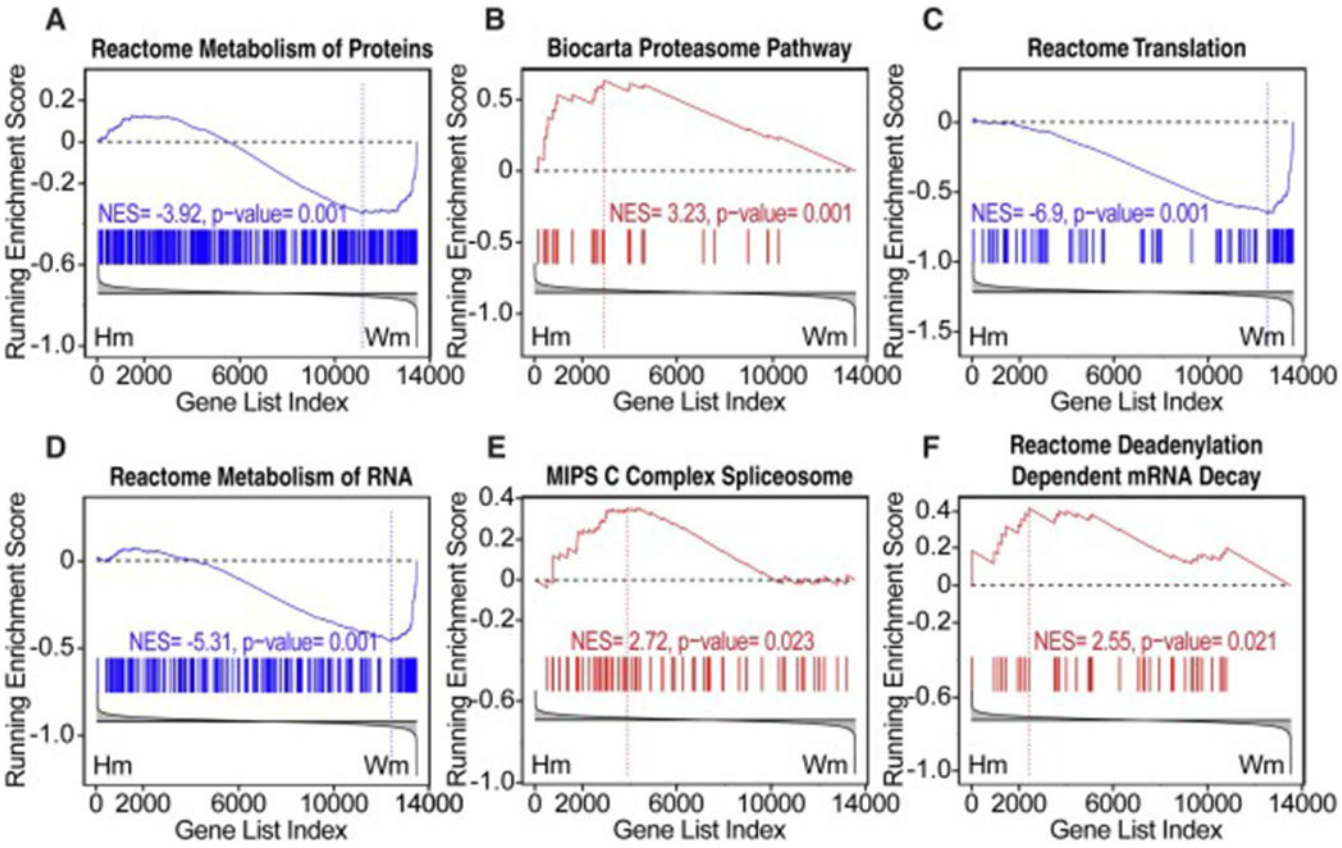

Fig. 6. Catabolic molecular environment in HIV Tg rats that self-administered METH under long-access conditions.

(**A-F**) Transcriptional evidence of impairments in metabolism of proteins and proteostasis **(A, B)**, lower protein synthesis **(C)** and alterations of RNA processing (**D-F**) in the mPFC in HIV Tg rats that self-administered METH under LgA conditions (Hm) compared with METH-self-administering WT rats (Wm).