Published in final edited form as:

Brain Behav Immun. 2018 July ; 71: 108–115. doi:10.1016/j.bbi.2018.04.004.

Recent Stimulant Use and Leukocyte Gene Expression In Methamphetamine Users with Treated HIV Infection

Adam W. Carrico, Ph.D.¹, Annesa Flentje, Ph.D.², Kord Kober, Ph.D.², Sulggi Lee, M.D., Ph.D.³, Peter Hunt, M.D.³, Elise D. Riley, Ph.D.³, Steven Shoptaw, Ph.D.⁴, Elena Flowers, Ph.D., R.N.^{2,5}, Samantha E. Dilworth, M.S.³, Savita Pahwa, M.D.¹, and Bradley E. Aouizerat, M.S., Ph.D.⁴

¹University of Miami School of Medicine

²University of California, San Francisco School of Nursing

³University of California, San Francisco School of Medicine

⁴University of California, Los Angeles School of Medicine

⁵University of California, San Francisco Institute for Human Genetics

⁶New York University College of Dentistry Bluestone Center for Clinical Research

Abstract

Stimulant use may accelerate HIV disease progression through biological and behavioral pathways. However, scant research with treated HIV-positive persons has examined stimulant-associated alterations in pathophysiologic processes relevant to HIV pathogenesis. In a sample of 55 HIV-positive, methamphetamine-using sexual minority men with a viral load less than 200 copies/mL, we conducted RNA sequencing to examine patterns of leukocyte gene expression in participants who had a urine sample that was reactive for stimulants ($n = 27$) as compared to those who tested non-reactive ($n = 28$). Results indicated differential expression of 32 genes and perturbation of 168 pathways in recent stimulant users. We observed statistically significant differential expression of single genes previously associated with HIV latency, cell cycle regulation, and immune activation in recent stimulant users (false discovery rate $p < 0.10$). Pathway analyses indicated enrichment for genes associated with inflammation, innate immune activation, neuroendocrine hormone regulation, and neurotransmitter synthesis. Recent stimulant users displayed concurrent elevations in plasma levels of tumor necrosis factor – alpha (TNF- α) but not interleukin 6 (IL-6). Further research is needed to examine the bio-behavioral mechanisms whereby stimulant use may contribute to HIV persistence and disease progression.

Corresponding Author: Adam W. Carrico, Ph.D. University of Miami Department of Public Health Sciences 1120 NW 14th St., Office 1005 Miami, FL 33136 Tel: 305-243-6947, a.carrico@miami.edu.

Publisher's Disclaimer: This is a PDF file of an unedited manuscript that has been accepted for publication. As a service to our customers we are providing this early version of the manuscript. The manuscript will undergo copyediting, typesetting, and review of the resulting proof before it is published in its final citable form. Please note that during the production process errors may be discovered which could affect the content, and all legal disclaimers that apply to the journal pertain.

Keywords

Cocaine; Gene Expression; HIV; Immune Activation; Inflammation; Methamphetamine; Reservoir

1. Introduction

Stimulant use may accelerate HIV disease progression via behavioral and biological mechanisms (Carrico, 2011; Salamanca et al., 2014). HIV-positive stimulant users are more likely to have difficulties with HIV disease management that contribute to elevated HIV viral load and hastened disease progression (Carrico, 2011; Carrico et al., 2011; Ellis et al., 2003). At the same time, recent findings from a cohort of HIV-positive sexual minority men receiving antiretroviral therapy (ART) indicate that those who engaged in more frequent stimulant use had 50% greater odds of clinical progression even after accounting for adherence and viral load (Carrico et al., 2014). Because most studies examining stimulant-associated immune dysregulation have relied on *in vitro* and animal models (Cabral, 2006), research with treated HIV-positive persons is needed to elucidate the biological pathways whereby stimulants may promote disease progression.

Sympathetic nervous system activation could partially mediate the immunomodulatory effects of stimulants in HIV, but findings from preclinical and human studies are mixed. Methamphetamine activates the sympathetic nervous system (Irwin et al., 2007; Makisumi et al., 1998) leading to elevated norepinephrine, which has been linked to greater HIV replication (Cole et al., 1998; Ironson et al., 2015). This is further supported by findings that sympathetic nervous activation and methamphetamine use may contribute to HIV persistence. For example, one prior study observed 3.9-fold greater rates of SIV replication at sites where lymph nodes juncture with catecholaminergic varicosities (Sloan et al., 2006). Among HIV-positive persons who were virally suppressed, greater sympathetic nervous system activation (measured by a shorter cardiac pre-ejection period) has also been associated with greater intracellular HIV RNA but not higher proviral HIV DNA (Hecht et al., 2017). Finally, recent findings with virally suppressed HIV-positive persons indicated that methamphetamine users had higher levels of proviral HIV DNA, but these results were not significant after adjusting for ART regimen (Massanella et al., 2015). Further research is needed to examine stimulant-associated alterations in biological processes relevant to HIV persistence.

Immune activation and inflammation have emerged as plausible, downstream mechanisms for the *in vivo* immunomodulatory effects of stimulants. In one laboratory-based study with HIV-negative men, cocaine infusion (versus placebo) was associated with decrements in monocyte expression of pro-inflammatory cytokine markers and decreased responsiveness of monocytes to lipopolysaccharide (Irwin et al., 2007). Cocaine-related reductions in monocyte expression of tumor necrosis factor - alpha (TNF- α) were partially attributable to greater increases in autonomic nervous system activation. Stimulant use could also contribute to immune dysregulation by upregulating indoleamine 2,3-dioxygenase (IDO). This is supported by a prior investigation that observed stimulant-associated elevations in neopterin and depleted tryptophan in HIV-positive persons after adjusting for ART

adherence (Carrico et al., 2008). Studies with virally suppressed, HIV-positive methamphetamine users also indicate that recent stimulant use is associated greater monocyte activation (Carrico et al., In Press) as well as CD4+ and CD8+ T-cell proliferation, activation, and exhaustion at rest compared to those who did not use methamphetamine (Massanella et al., 2015). More research is clearly needed to elucidate the mechanisms of stimulant-associated immune dysregulation in treated HIV.

One important consideration is that stimulant use may influence multiple biological pathways and trigger opposing biological processes initiated to maintain homeostasis, obscuring the association of stimulant use with plasma immune markers. Gene expression is a transcriptomic approach that allows for exploration of multiple stimulant-associated alterations in leukocyte signaling, potentially providing greater clarity regarding the mechanisms of stimulant-associated biological alterations in treated HIV. For example, one *in vitro* study observed treatment of dendritic cells with methamphetamine led to altered gene expression for pathways governing chemokine regulation, cytokine production, signal transduction mechanisms, apoptosis, and cell cycle regulation (Mahajan et al., 2006).

The present study examined the association of recent stimulant use with leukocyte gene expression in a sample of HIV-positive, methamphetamine-using sexual minority men who were receiving effective ART. We hypothesized that participants who provided a urine sample that was reactive for stimulants (i.e., Stimulant Tox+) would display differential gene expression across multiple biological pathways relevant to HIV pathogenesis as compared to those who tested non-reactive for recent stimulant use (i.e., Stimulant Tox-). We also hypothesized that recent stimulant users would display elevated plasma levels of TNF- α and interleukin 6 (IL-6).

2. Methods

HIV-positive, methamphetamine-using sexual minority men were recruited for a randomized controlled trial from substance abuse treatment programs, HIV medical clinics, AIDS service organizations, the community, and referrals from active participants (Carrico et al., 2016). At an in-person screening visit, participants completed signed informed consent that included consent for specimen banking. All enrolled participants met the following inclusion criteria: 1) 18 years of age or older; 2) a sexual minority man; 3) documentation of HIV-positive serostatus (i.e., letter of diagnosis or ART medications other than Truvada matched to photo identification); and 4) provide a urine or hair sample at the screening visit that was reactive for methamphetamine. For the purposes of this study, participants were restricted to individuals who had an HIV viral load < 200 copies/mL.

Enrolled participants completed a separate baseline assessment approximately one week later that included a detailed battery of psychosocial measures, a urine sample for on-site toxicology testing, and peripheral venous blood sample to measure HIV disease markers. In addition, participants also provided two $\times$ 10 mL EDTA and two $\times$ 2.5 mL PAXgene® Blood RNA tubes (Qiagen, Inc.) for specimen banking. This study was approved by the Institutional Review Boards for the University of California, San Francisco as well as the

University of Miami and Northwestern University. A certificate of confidentiality was obtained from the National Institute on Drug Abuse.

2.1. Measures

2.1.1. Demographics and health status—Participants completed a demographic questionnaire assessing age, race, ethnicity, and health-related factors such as current ART regimen.

2.1.2. ART adherence and persistence—Participants rated their adherence to each ART medication during the past 30 days using the visual analogue scale (Walsh et al., 2002), which was averaged to calculate the percentage of medications taken. Participants also indicated if they have experienced a treatment interruption, a period of two days or more in the past six months where all HIV medications were stopped without guidance from an HIV primary care provider. Participants without a treatment interruption (1) were compared to those with a treatment interruption (0) as a measure of ART persistence.

2.1.3. Psychiatric comorbidities—The Addiction Severity Index (ASI) was administered to assess the severity of alcohol and other substance use (McLellan et al., 1992). Depressive symptom severity was assessed using the 20-item Centers for the Epidemiologic Study - Depression (CES-D) scale (Radloff, 1977). Post-Traumatic Stress Disorder (PTSD) symptoms were measured with the PTSD Checklist – Civilian (PCL-C) version (Wilkins et al., 2011). Because depression and PTSD may have immunomodulatory effects, we examined whether they were potential confounders by comparing the Stimulant Tox+ and Stimulant Tox– groups.

2.1.4. HIV disease markers—HIV viral load testing was performed to detect plasma HIV RNA using the Abbott Real Time HIV-1 assay (Abbott Molecular, Inc.; Des Plaines, IL). This assay has a lower limit of detection of 40 copies/mL. CD4+ T-cell count was measured with whole blood using flow cytometry, and assays were performed by Quest Diagnostics.

2.1.5. On-site urine screening—Urine samples were collected for on-site toxicology screening using the iCup (Redwood Biotech, Inc.; Santa Rosa, CA), which is capable of detecting stimulant use within the past 72 hours. Results were used to identify participants who tested reactive for recent methamphetamine or cocaine use (i.e., Stimulant Tox+) versus those who were non-reactive for both (i.e., Stimulant Tox–). Among those who were Stimulant Tox+, all but one were reactive for methamphetamine. Given the relatively rapid temporal dynamics of leukocyte gene expression, we examined recent stimulant use as the primary predictor.

2.1.6. Plasma pro-inflammatory cytokines—Plasma levels of TNF- α and IL-6 were determined by the use of Human Quantikine Immunoassay using undiluted samples (R&D Systems, Minneapolis, MN) following the manufacturer's instructions. Results were log₁₀ transformed.

2.1.7. RNA sample preparation, sequencing, and data management—Total RNA was isolated from the PAXgene® tubes using the PAXgene® Blood miRNA Kit (Qiagen, Inc.). Total RNA for 62 unique samples was sent for library preparation and sequencing at the Vincent J. Coates Genomics Sequencing Laboratory at the University of California, Berkeley. Prior to library preparation, total RNA was treated with Ribo-Zero rRNA Removal Kit (Human, Mouse, Rat; Illumina Inc., San Diego, CA) to deplete cytoplasmic ribosomal RNA (O'Neil et al., 2013). RNA was then prepared for sequencing on an Apollo 324™ with PrepX™ RNAseq Library Prep reagents (WaferGen Biosystems, Fremont, CA) following the manufacturers protocol. Thirteen cycles of PCR amplification were used for single 6 base pair index addition and library fragment enrichment. Prepared libraries were then quantified on a Roche Light Cycler 480II (Roche Diagnostics Corp., Indianapolis, IN) using KAPA Illumina library quantitative PCR reagents (Roche Diagnostics Corp., Indianapolis, IN).

Sequencing was done on an Illumina HiSeq 4000 apparatus (Illumina Inc., San Diego, CA). Sixty-two samples were sequenced, with second samples from 10 library preparations included as technical replicates, for a total of 72 sequenced samples. Eight lanes were sequenced with 9 samples multiplexed per lane for 100 cycles of paired-end reads with a 1% PhiX v3 control library spike (Illumina Inc., San Diego, CA). Post-sequencing basecall files (bclfiles) were demultiplexed and converted into FASTQ file format using the bcl2fastq v2.17 software (Illumina Inc., San Diego, CA). Data was then posted and retrieved from a secured FTP site hosted by the core facility.

RNA sequencing (RNAseq) data processing was performed based on recently delineated best practices (Conesa et al., 2016; Kukurba and Montgomery, 2015). The fastq_illumina_filter (http://cancan.cshl.edu/labmembers/gordon/fastq_illumina_filter/) was used to retain only high quality reads. Illumina adapters and leading or trailing low quality bases were removed and reads with an the average quality per base below 15 in a 4-base sliding window or below a minimum length of 36 bases were removed using Trimmomatic (Bolger et al., 2014). Reads were inspected with FASTQC (<http://www.bioinformatics.babraham.ac.uk/projects/fastqc/>). An unequal frequency of alleles was observed at the beginning of the reads across the majority of the libraries, so 12 bases were trimmed from all reads. Reads were again inspected with FASTQC with improved allele frequencies. The reference genome was prepared using the GRCh38 assembly (gencode.v24.GRCh38.p5.fa). Transcriptome annotations were obtained from the Gencode v24 primary assembly (gencode.v24.primary_assembly.annotation.gtf). Trimmed reads were aligned to the reference genome and annotation using the STAR aligner (Dobin et al., 2013).

Output SAM files were validated using ValidateSam. Read groups were added to the SAM file using the Picard tool AddOrReplaceReadGroups. Sorted files were inspected using RNA-SeQC (DeLuca et al., 2012). Abundance of RNA were estimated from aligned reads using featureCounts (Liao et al., 2014). Replicate count data were processed in edgeR (Robinson et al., 2010). Ensembl (<http://www.ensembl.org>) transcripts were annotated with Entrez gene ID and symbol (Maglott et al., 2011). Replication quality and repeatability was measured as Pearson correlations between the ten sample replicates pairs.

Lowly expressed tags were filtered out by retaining only those with 1 read per million in at least 27 samples. Count estimates were normalized with the trimmed means of M (TMM) values method (Robinson and Oshlack, 2010). TMM normalization was applied to the dataset in edgeR using calcNormFactors.

One sample from each replicate pair was excluded using a coin-flip, leaving a total of 62 samples for the analysis. Data exploration was performed with multi-dimensional scaling (MDS) plots for all samples to identify sample outliers and potential batch effects due to technical artifacts (e.g., RNA quality, processing laboratory, technician, sequencing lane). Four samples that were outliers in the MDS plots and three samples with an HIV viral load > 200 copies/mL were excluded, leaving 55 samples for further analysis. Surrogate Variable Analysis (SVA) was used to identify variation contributing to heterogeneity in the sample (e.g., batch effects) not due to the variable of interest, Stimulant Tox group membership (Leek and Storey, 2007). To identify which surrogate variables to include, all surrogate variables were tested for correlation with the phenotype, RNA integrity number (RIN), laboratory technician, sequencing lane, and library size. Significance was assessed at a nominal p-value of 0.05. Any surrogate variable which correlated with the phenotype ($p < 0.10$) was excluded.

2.2. Statistical Analyses

Independent samples t-tests and chi-square analyses were utilized to compare the Stimulant Tox+ and Stimulant Tox- groups on demographics, health status, psychiatric factors, and log₁₀ plasma pro-inflammatory cytokine levels. Although there were no group differences in ART regimen, SVA gene expression analyses controlled for unmeasured sources of confounding.

Differential expression was determined under a variance modeling strategy that addresses the over-dispersion observed in gene expression count data in edgeR (Landau and Liu, 2013). A standard software package, edgeR, is widely used for differential gene expression analysis and performs well relative to other strategies (Rapaport et al., 2013; Soneson and Delorenzi, 2013). We followed best practices that have been previously articulated (Anders et al., 2013; Love et al., 2015). For this analysis, the overall dispersion as well as the gene-wise and tag-wise dispersion were estimated using general linear models (GLM) estimated using the Cox-Reid (CR)-adjusted likelihood method (Cox and Reid, 1987; McCarthy et al., 2012). Two of the twelve surrogate variables identified by SVA were correlated with Stimulant Tox group membership at $p < 0.10$ and were excluded. Gene-wise negative-binomial GLMs that adjusted for the 10 surrogate variables were fit. Differences between the Stimulant Tox+ and Stimulant Tox- groups were tested using likelihood ratio tests ($p < 0.05$). We adjusted for multiple hypothesis testing through estimation of the false discovery rate (FDR) under the Benjamini-Hochberg procedure with a significance cut-off of 10% with no minimal log-fold change (Benjamini and Hochberg, 1995; Hochberg and Benjamini, 1990). However, we report the unadjusted p-values for genes relevant to TNF- α as posthoc probing to characterize biological pathways underlying significantly elevated plasma levels in recent stimulant users.

Sequence loci data were annotated with Entrez gene IDs. The gene names were annotated using the HUGO Gene Nomenclature Committee resource database (Gray et al., 2013). In order to discover differences between Stimulant Tox+ and Stimulant Tox– groups in perturbations among genes that operate together in higher orders (i.e., pathways), we performed a competitive analysis of differentially perturbed pathways using the R package “GAGE” (Luo et al., 2009). A competitive approach tests if the gene set of a pathway is differentially expressed compared to the set of complementary genes (Emmert-Streib and Glazko, 2011; Goeman and Buhlmann, 2007). For GAGE pathway analyses, raw count data were preprocessed and normalized *de novo* following the author’s recommended instructions. Only loci annotated with ENTREZ IDs were included in the analysis.

Signaling pathway impact analysis (SPIA, Tarca et al., 2009), which integrates patterns of perturbation with the topology of the pathway, was used to identify pathway perturbations that differed between Stimulant Tox+ and Stimulant Tox– groups. Only loci annotated with ENTREZ IDs and unadjusted *p* values <0.001 in the differential gene expression analyses were retained for consideration in SPIA analyses.

3. Results

As shown in Table 1, the Stimulant Tox+ and Stimulant Tox– groups were generally comparable with regard to demographic and health-related factors, including no significant differences in ART regimen, ART adherence and persistence, prevailing CD4+ T-cell count, and the proportion with an HIV viral load below the limit of assay detection (less than 40 copies/mL). On average, Stimulant Tox+ participants reported 2.68 times more days using methamphetamine in the past 30 days (10.7 versus 4.0). Those who were Stimulant Tox+ displayed significantly higher log₁₀ TNF-α levels (1.7 versus 1.6). Although log₁₀ IL-6 were also higher in recent stimulant users (0.4 versus 0.3), this did not reach statistical significance (*p* = 0.10).

Mean RIN was 7.2 (min. 6.3), while median library size was 14,060,000 reads; 20,426 transcripts were retained with at least 1 read per million in 10 samples from the total of 60,725 GENCODE transcript targets. We found high replicability for the count data with a mean within-replicate Pearson correlation coefficient of <0.9999. No batch effects were identified for sample preparation order, RIN value, RNA processing date or technician, or sequencing lane in either the first two dimensions of MDS plots. In line with values reported in other clinical datasets (McCarthy et al., 2012), the overall dispersion was observed to be 0.2381 (Biological Coefficient of Variation = 0.4879).

As shown in Table 2, 32 genes were differentially expressed in the Stimulant Tox+ group as compared to the Stimulant Tox– group. Results suggest that recent stimulant use was associated with upregulation of genes regulating HIV latency and survival of HIV-infected cells (i.e., *PDL1*, *KAT2B*, *FCGR2A*, *LGALS3*, *HIST1H4A*), cell cycle regulation (e.g., *FOXO4*, *MED21*), and immune activation (i.e., *FCGR1CP*, *FCGR1B*, *RBM38*, *FCGR1A*). We also observed downregulation of *FAT1* and upregulation of *FUT8*, both of which are relevant to cancer. Results indicated downregulation of *MAN2A2*, *GAA*, and *FAM210B*, which are relevant to metabolic functioning (e.g., glycan synthesis and glycogen

degradation). Finally, there was some evidence for differential expression of genes governing inflammation, neural and hormonal functioning, and cardiovascular risk.

In order to explore the plausible mechanisms underlying elevated TNF- α levels in plasma, we conducted posthoc assessment of genes relevant to the TNF superfamily. We observed upregulation of *TNFSF13B* (Entrez Gene ID = 10673; Fold Change = 0.35; $p_{\text{unadjusted}}$ = 0.003), *TNFSF10* (Entrez Gene ID = 8743; Fold Change = 0.24; $p_{\text{unadjusted}}$ = 0.008), *TNFAIP6* (Entrez Gene ID = 7130; Fold Change = 0.46; $p_{\text{unadjusted}}$ = 0.033), and *TNFRSF1A* (Entrez Gene ID = 7132; Fold Change = 0.11; $p_{\text{unadjusted}}$ = 0.036). These genes underlie B-cell stimulation, apoptosis, innate immune activation, and receptor expression relevant to TNF- α . There was no differential expression of *TNF* (Entrez Gene ID = 7124; Fold Change = 0.06; $p_{\text{unadjusted}}$ = 0.79), which is linked to TNF- α production.

Evidence of two-directional perturbation of 168 KEGG pathways was observed (Supplemental Table 1). Table 3 summarizes those pathways with greatest relevance to HIV pathogenesis and neuropsychiatric disorders. Findings provided further support for two-directional perturbation of pathways relevant to HIV latency in recent stimulant users. Pathways relevant to HIV latency included: pathways in cancer, cell cycle, arachidonic acid metabolism, apoptosis, and the mTOR signaling pathway. Results also provided support for two-directional perturbation of pathways relevant to inflammation including: cytokine-cytokine receptor interaction, chemokine signaling pathway, MAPK signaling pathway, Jak-STAT signaling, and the Fc epsilon RI signaling pathway. We also observed two-directional perturbation of pathways relevant to the innate and acquired immune activation such as antigen processing and presentation, toll-like receptor signaling, natural killer cell cytotoxicity, and Fc gamma R-mediated phagocytosis, and T-cell activation. Two-directional perturbation of pathways linked to neuroendocrine hormone regulation (i.e., neuroactive ligand-receptor interaction and autoimmune thyroid disease) as well as synthesis of catecholamines (i.e., tyrosine metabolism) and serotonin (i.e., tryptophan metabolism) were also observed. These pathways are directly relevant the regulation of immunomodulatory neuroendocrine hormones as well as the etiology of neuropsychiatric disorders. Finally, we observed two-directional perturbation of pathways in cancer.

4. Discussion

The results of the present study provide support for stimulant-associated alterations in patterns of gene expression relevant to HIV pathogenesis in a sample of methamphetamine users with treated HIV. Because this was a sample comprised entirely of methamphetamine users, this study examined dose-response associations of recent stimulant use. Below we briefly discuss these complex associations of recent stimulant use with alterations of gene expression patterns across multiple biological systems.

Recent stimulant use was associated with patterns of differential expression in genes previously associated with HIV persistence and residual immune dysregulation during ART suppression. Recent stimulant use was associated with upregulation of *FCGR2A*, which encodes for a marker (i.e., CD32a) that is expressed on CD4⁺ T-cells, potentially marking replication-competent cells (Descours et al., 2017). Recent stimulant users also

demonstrated upregulation of *PDL1*, encoding the ligand for an immune exhaustion marker (i.e., programmed cell death-1 ligand) which is another putative cell surface marker identifying CD4+ T-cells with replication-competent HIV (Banga et al., 2016). Consistent with prior research (Massanella et al., 2015), our findings highlight that stimulant use may contribute to HIV persistence by facilitating HIV latency and persistent, low level HIV replication. Our results also suggest that stimulant use could lead to greater HIV persistence by inhibiting apoptosis of HIV-infected cells and metabolic dysregulation leading to expansion of the HIV reservoir in CD4+ T-cells (Chomont et al., 2009). Further research examining these biological pathways whereby stimulant use may contribute to HIV persistence is needed to inform the development of novel HIV cure interventions.

There was also some evidence that recent stimulant use is associated with dysregulation of gene expression patterns relevant to immunosurveillance. For example, recent stimulant users displayed downregulation of a gene relevant to tumor suppression (i.e., *FAT1*) and concurrent upregulation a gene that may promote metastasis (i.e., *FUT8*). These results are consistent with prior research where virally suppressed methamphetamine users displayed impaired proliferative responses after stimulation with various pathogens compared to those who did not use methamphetamine (Massanella et al., 2015). More research is warranted to examine the specific mechanisms that may underlie stimulant-associated impairments in immunosurveillance, including oncogenic viruses that are implicated in AIDS-related cancers.

Findings also provide some support for stimulant-associated alterations in gene expression relevant to immune activation and inflammation. We observed upregulation of multiple genes relevant to the Fc region of antibodies that are crucial for coordinating immune activation and supporting antibody-dependent cytotoxicity. As evidenced in the pathway analyses, this pattern of stimulant-associated increases in immune activation could contribute to greater inflammation. For example, alterations in Fc Epsilon RI signaling are linked to *TNF* transcription (Kraft and Bieber, 2001). We also observed upregulation of genes governing B-cell stimulation, apoptosis, innate immune activation, and receptor expression on immune cells relevant to TNF superfamily in recent stimulant users. This may partially explain why recent stimulant users in the present study displayed higher plasma levels of TNF- α . Findings are consistent with prior research in HIV-negative persons where reductions in monocyte expression of TNF- α following cocaine infusion were partially attributable to greater increases in autonomic nervous system activation (Irwin et al., 2007).

Consistent with prior *in vitro* research using dendritic cells (Mahajan et al., 2006), we observed stimulant-associated dysregulation of pathways governing chemokine regulation, cytokine production, apoptosis, and cell cycle regulation in leukocytes. Our findings also indicated that recent stimulant use was associated with two-directional, differential expression of pathways governing adhesion, trafficking, and cytotoxic activity of leukocytes. These results lend further support to the conclusion that stimulant use may disrupt immunosurveillance in treated HIV, which could have important implications for responding to novel pathogens, terminating inflammation, and eradicating neoplasias.

Finally, results of pathway analyses provide some evidence for stimulant-associated alterations in neuroendocrine hormone regulation and neurotransmitter synthesis. The acute effects of methamphetamine are attributable to sympathetic nervous system activation (Makisumi et al., 1998), resulting in the release of catecholamines such as norepinephrine at sympathetic nerve terminals and increased dopamine production in the central nervous system (Wilson et al., 1996). In addition, the hypothalamic-pituitary-adrenal (HPA) and hypothalamic-pituitary-thyroidal (HPT) axes are thought to be dysregulated in chronic methamphetamine users (Carrico et al., 2018; Goeders, 2002; Li et al., 2013). This was observed in pathway analyses indicating possible dysregulation of the HPA and HPT axes. Recent stimulant use was also associated with two-directional, differential expression of pathways for metabolism of amino acid precursors for serotonin (i.e., tryptophan) as well as catecholamines such as norepinephrine and dopamine (i.e., tyrosine). These results are consistent with prior cross-sectional research where more frequent stimulant use was associated with depleted tryptophan in plasma among HIV-positive persons on ART (Carrico et al., 2008). Future research should determine whether stimulant-induced neuroendocrine dysregulation and perturbations in neurotransmitter synthesis mediate immunologic changes relevant to HIV pathogenesis.

A common aspect of studies of methamphetamine use and viral dynamics is the influence of ART adherence. In our study, we were only able to assess adherence using self-report measures and by restricting participants to those with an HIV RNA < 200 copies/mL. It is plausible that the gene expression changes observed here reflect transcriptional changes as a result of inadequate viral suppression rather than a direct effect of methamphetamine per se. Nonetheless, our analyses highlight several intriguing patterns suggestive of distinct inflammatory and latency-associated pathways that need to be further investigated in cohort studies using more rigorous measures of ART adherence in people who use methamphetamine.

This study has other limitations that deserve mention. First, recent stimulant use measured in urine reflects any use during the past 72 hours, and it is plausible that differential patterns of leukocyte gene expression reflect different phases of use like intoxication or withdrawal. Future research should assess methamphetamine and cocaine concentrations through quantitative toxicology measures. Second, all participants enrolled in this study were biologically confirmed methamphetamine users and further research is needed to examine differential patterns of leukocyte gene expression in comparison to non-users. Third, unhealthy alcohol use and other psychiatric comorbidities may independently influence gene expression. In fact, another recent study from our team led by Flentje and colleagues (2018) demonstrates that sexual minority stress processes are associated with differential leukocyte gene expression after controlling for recent stimulant use. Fourth, research is needed to examine differential gene expression as a function of chronic patterns of stimulant use, different route(s) of stimulant administration, and distinct typologies of polysubstance use. Future research should also measure other psychiatric and medical comorbidities in relation to gene expression in treated HIV. Finally, it is plausible that there are pharmacodynamic interactions between stimulant use and specific ART medications. Further research is needed to examine the influence of specific ART regimens as moderators.

Despite these limitations, results from this study provide some of the first evidence for potential stimulant-associated alterations of gene expression patterns relevant to HIV pathogenesis. These findings may help inform efforts to better understand potential biobehavioral mechanisms by which stimulant use may contribute to HIV persistence, dysregulate immunosurveillance, and disrupt neuroimmune signaling in treated HIV.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

Acknowledgments

This project was supported by the National Institute on Drug Abuse (R01-DA033854; Woods, Carrico, and Moskowitz, PIs). Additional funding was provided by pilot awards from the University of California, San Francisco Center for AIDS Research (P30-AI027763; Volberding, PI) and the National Institute on Drug Abuse (P50-009253; Guydish, PI; T32-DA007250; Sorensen, PI; K23DA039800; Flentje, PI). Additional support for assays of plasma cytokines was provided by the Miami Center for AIDS Research (P30-AI073961; Pahwa, PI). This work used the Vincent J. Coates Genomics Sequencing Laboratory at UC Berkeley, supported by NIH S10 OD018174 Instrumentation Grant. We would also like to thank Dr. Teri Leigler for her support of this project through the University of California, San Francisco Center for AIDS Research's Virology Core (P30-AI027763). The team would like to express our gratitude to multiple staff who have contributed time and effort to the successful execution of this project including: Justin Lagana-Jackson, Paul Cotten, and Lara Coffin. Finally, we are grateful to the study participants who placed a great deal of trust in our team to collect and manage biological specimens for this project.

References

- Anders S, McCarthy DJ, Chen Y, Okoniewski M, Smyth GK, Huber W, Robinson MD. Count-based differential expression analysis of RNA sequencing data using R and Bioconductor. *Nat Protoc.* 2013; 8:1765–1786. [PubMed: 23975260]
- Banga R, Procopio FA, Noto A, Pollakis G, Cavassini M, Ohmiti K, Corpataux JM, de Leval L, Pantaleo G, Perreau M. PD-1(+) and follicular helper T cells are responsible for persistent HIV-1 transcription in treated aviremic individuals. *Nat Med.* 2016; 22:754–761. [PubMed: 27239760]
- Benjamini Y, Hochberg Y. Controlling the False Discovery Rate: A Practical and Powerful Approach to Multiple Testing. *Journal of the Royal Statistical Society Series B (Methodological).* 1995; 57:289–300.
- Bolger AM, Lohse M, Usadel B. Trimmomatic: a flexible trimmer for Illumina sequence data. *Bioinformatics.* 2014; 30:2114–2120. [PubMed: 24695404]
- Cabral GA. Drugs of abuse, immune modulation, and AIDS. *J Neuroimmune Pharmacol.* 2006; 1:280–295. [PubMed: 18040805]
- Carrico AW. Substance use and HIV disease progression in the HAART era: implications for the primary prevention of HIV. *Life Sci.* 2011; 88:940–947. [PubMed: 20934437]
- Carrico AW, Cherenack EM, Roach ME, Riley ED, Oni O, Dilworth SE, Shoptaw S, Hunt P, Roy S, Pallikkuth S, Pahwa S. Substance-associated elevations in monocyte activation among methamphetamine users with treated HIV infection. *AIDS.* In Press.
- Carrico AW, Jain J, Discepolo MV, Olem D, Andrews R, Woods WJ, Neilands TB, Shoptaw S, Gomez W, Dilworth SE, Moskowitz JT. A community-engaged randomized controlled trial of an integrative intervention with HIV-positive, methamphetamine-using men who have sex with men. *BMC Public Health.* 2016; 16:673. [PubMed: 27476110]
- Carrico AW, Johnson MO, Morin SF, Remien RH, Riley ED, Hecht FM, Fuchs D. Stimulant use is associated with immune activation and depleted tryptophan among HIV-positive persons on anti-retroviral therapy. *Brain Behav Immun.* 2008; 22:1257–1262. [PubMed: 18703133]
- Carrico AW, Riley ED, Johnson MO, Charlebois ED, Neilands TB, Remien RH, Lightfoot MA, Steward WT, Weinhardt LS, Kelly JA, Rotheram-Borus MJ, Morin SF, Chesney MA. Psychiatric risk factors for HIV disease progression: the role of inconsistent patterns of antiretroviral therapy utilization. *J Acquir Immune Defic Syndr.* 2011; 56:146–150. [PubMed: 21116186]

- Carrico AW, Rodriguez VJ, Jones DL, Kumar M. Short circuit: Disaggregation of adrenocorticotrophic hormone and cortisol levels in HIV-positive, methamphetamine-using men who have sex with men. *Hum Psychopharmacol*. 2018; 33
- Carrico AW, Shoptaw S, Cox C, Stall R, Li X, Ostrow DG, Vlahov D, Plankey MW. Stimulant use and progression to AIDS or mortality after the initiation of highly active antiretroviral therapy. *J Acquir Immune Defic Syndr*. 2014; 67:508–513. [PubMed: 25271387]
- Chomont N, El-Far M, Ancuta P, Trautmann L, Procopio FA, Yassine-Diab B, Boucher G, Boulassel MR, Ghattas G, Brechley JM, Schacker TW, Hill BJ, Douek DC, Routy JP, Haddad EK, Sekaly RP. HIV reservoir size and persistence are driven by T cell survival and homeostatic proliferation. *Nat Med*. 2009; 15:893–900. [PubMed: 19543283]
- Cole SW, Korin YD, Fahey JL, Zack JA. Norepinephrine accelerates HIV replication via protein kinase A-dependent effects on cytokine production. *J Immunol*. 1998; 161:610–616. [PubMed: 9670934]
- Conesa A, Madrigal P, Tarazona S, Gomez-Cabrero D, Cervera A, McPherson A, Szczesniak MW, Gaffney DJ, Elo LL, Zhang X, Mortazavi A. A survey of best practices for RNA-seq data analysis. *Genome Biol*. 2016; 17:13. [PubMed: 26813401]
- Cox D, Reid N. Parameter orthogonality and approximate conditional inference. *J Roy Stat Soc Ser B Method*. 1987; 49:1–39.
- DeLuca DS, Levin JZ, Sivachenko A, Fennell T, Nazaire MD, Williams C, Reich M, Winckler W, Getz G. RNA-SeQC: RNA-seq metrics for quality control and process optimization. *Bioinformatics*. 2012; 28:1530–1532. [PubMed: 22539670]
- Descours B, Petitjean G, Lopez-Zaragoza JL, Bruel T, Raffel R, Psomas C, Reynes J, Lacabartz C, Levy Y, Schwartz O, Lelievre JD, Benkirane M. CD32a is a marker of a CD4 T-cell HIV reservoir harbouring replication-competent proviruses. *Nature*. 2017; 543:564–567. [PubMed: 28297712]
- Dobin A, Davis CA, Schlesinger F, Drenkow J, Zaleski C, Jha S, Batut P, Chaisson M, Gingeras TR. STAR: ultrafast universal RNA-seq aligner. *Bioinformatics*. 2013; 29:15–21. [PubMed: 23104886]
- Ellis RJ, Childers ME, Cherner M, Lazzaretto D, Letendre S, Grant I, Group, H.I.V.N.R.C. Increased human immunodeficiency virus loads in active methamphetamine users are explained by reduced effectiveness of antiretroviral therapy. *J Infect Dis*. 2003; 188:1820–1826. [PubMed: 14673760]
- Emmert-Streib F, Glazko GV. Pathway analysis of expression data: deciphering functional building blocks of complex diseases. *PLoS Comput Biol*. 2011; 7:e1002053. [PubMed: 21637797]
- Flentje A, Kober KM, Carrico AW, Neilands TB, Flowers E, Heck NC, Aouizerat BE. Minority stress and leukocyte gene expression in sexual minority men living with treated HIV infection. *Brain Behav Immun*. 2018
- Goeders NE. The HPA axis and cocaine reinforcement. *Psychoneuroendocrinology*. 2002; 27:13–33. [PubMed: 11750768]
- Goeman JJ, Buhlmann P. Analyzing gene expression data in terms of gene sets: methodological issues. *Bioinformatics*. 2007; 23:980–987. [PubMed: 17303618]
- Gray KA, Daugherty LC, Gordon SM, Seal RL, Wright MW, Bruford EA. Genenames.org: the HGNC resources in 2013. *Nucleic Acids Res*. 2013; 41:D545–552. [PubMed: 23161694]
- Hecht, F., Moran, P., Mendes, WB., Cockerham, L., Hartogensis, W., Bacchetti, P., Cameron, P., Chang, J., Deeks, S., Lewin, S. Stress increases HIV transcription in HIV-infected individuals on anti-retroviral therapy: Implications for biomarkers of HIV persistence; International Conference on HIV Science; Paris, France. 2017.
- Hochberg Y, Benjamini Y. More powerful procedures for multiple significance testing. *Stat Med*. 1990; 9:811–818. [PubMed: 2218183]
- Ironson G, O'Cleirigh C, Kumar M, Kaplan L, Balbin E, Kelsch CB, Fletcher MA, Schneiderman N. Psychosocial and Neurohormonal Predictors of HIV Disease Progression (CD4 Cells and Viral Load): A 4 Year Prospective Study. *AIDS Behav*. 2015; 19:1388–1397. [PubMed: 25234251]
- Irwin MR, Olmos L, Wang M, Valladares EM, Motivala SJ, Fong T, Newton T, Butch A, Olmstead R, Cole SW. Cocaine dependence and acute cocaine induce decreases of monocyte proinflammatory cytokine expression across the diurnal period: autonomic mechanisms. *J Pharmacol Exp Ther*. 2007; 320:507–515. [PubMed: 17068203]
- Kraft S, Bieber T. Fc epsilon ri-mediated activation of transcription factors in antigen-presenting cells. *Int Arch Allergy Immunol*. 2001; 125:9–15. [PubMed: 11385283]

- Kukurba KR, Montgomery SB. RNA Sequencing and Analysis. Cold Spring Harb Protoc. 2015; 2015:951–969. [PubMed: 25870306]
- Landau WM, Liu P. Dispersion estimation and its effect on test performance in RNA-seq data analysis: a simulation-based comparison of methods. PLoS One. 2013; 8:e81415. [PubMed: 24349066]
- Leek JT, Storey JD. Capturing heterogeneity in gene expression studies by surrogate variable analysis. PLoS Genet. 2007; 3:1724–1735. [PubMed: 17907809]
- Li SX, Yan SY, Bao YP, Lian Z, Qu Z, Wu YP, Liu ZM. Depression and alterations in hypothalamic-pituitary-adrenal and hypothalamic-pituitary-thyroid axis function in male abstinent methamphetamine abusers. Hum Psychopharmacol. 2013; 28:477–483. [PubMed: 23913817]
- Liao Y, Smyth GK, Shi W. featureCounts: an efficient general purpose program for assigning sequence reads to genomic features. Bioinformatics. 2014; 30:923–930. [PubMed: 24227677]
- Love MI, Anders S, Kim V, Huber W. RNA-Seq workflow: gene-level exploratory analysis and differential expression. F1000Res. 2015; 4:1070. [PubMed: 26674615]
- Luo W, Friedman MS, Shedden K, Hankenson KD, Woolf PJ. GAGE: generally applicable gene set enrichment for pathway analysis. BMC Bioinformatics. 2009; 10:161. [PubMed: 19473525]
- Maglott D, Ostell J, Pruitt KD, Tatusova T. Entrez Gene: gene-centered information at NCBI. Nucleic Acids Res. 2011; 39:D52–57. [PubMed: 21115458]
- Mahajan SD, Hu Z, Reynolds JL, Aalinkeel R, Schwartz SA, Nair MP. Methamphetamine modulates gene expression patterns in monocyte derived mature dendritic cells: implications for HIV-1 pathogenesis. Mol Diagn Ther. 2006; 10:257–269. [PubMed: 16884330]
- Makisumi T, Yoshida K, Watanabe T, Tan N, Murakami N, Morimoto A. Sympatho-adrenal involvement in methamphetamine-induced hyperthermia through skeletal muscle hypermetabolism. Eur J Pharmacol. 1998; 363:107–112. [PubMed: 9881575]
- Massanella M, Gianella S, Schrier R, Dan JM, Perez-Santiago J, Oliveira MF, Richman DD, Little SJ, Benson CA, Daar ES, Dube MP, Haubrich RH, Smith DM, Morris SR. Methamphetamine Use in HIV-infected Individuals Affects T-cell Function and Viral Outcome during Suppressive Antiretroviral Therapy. Sci Rep. 2015; 5:13179. [PubMed: 26299251]
- McCarthy DJ, Chen Y, Smyth GK. Differential expression analysis of multifactor RNA-Seq experiments with respect to biological variation. Nucleic Acids Res. 2012; 40:4288–4297. [PubMed: 22287627]
- McLellan AT, Kushner H, Metzger D, Peters R, Smith I, Grissom G, Pettinati H, Argeriou M. The Fifth Edition of the Addiction Severity Index. J Subst Abuse Treat. 1992; 9:199–213. [PubMed: 1334156]
- O’Neil D, Glowatz H, Schlumpberger M. Ribosomal RNA depletion for efficient use of RNA-seq capacity. Curr Protoc Mol Biol. 2013;19. Chapter 4, Unit 4. [PubMed: 23821444]
- Radloff LS. The CES-D scale: A self-report depression scale for research in the general population. Applied Psychological Measurement. 1977; 1:385–341.
- Rapaport F, Khanin R, Liang Y, Pirun M, Krek A, Zumbo P, Mason CE, Socci ND, Betel D. Comprehensive evaluation of differential gene expression analysis methods for RNA-seq data. Genome Biol. 2013; 14:R95. [PubMed: 24020486]
- Robinson MD, McCarthy DJ, Smyth GK. edgeR: a Bioconductor package for differential expression analysis of digital gene expression data. Bioinformatics. 2010; 26:139–140. [PubMed: 19910308]
- Robinson MD, Oshlack A. A scaling normalization method for differential expression analysis of RNA-seq data. Genome Biol. 2010; 11:R25. [PubMed: 20196867]
- Salamanca SA, Sorrentino EE, Nosanchuk JD, Martinez LR. Impact of methamphetamine on infection and immunity. Front Neurosci. 2014; 8:445. [PubMed: 25628526]
- Sloan EK, Tarara RP, Capitanio JP, Cole SW. Enhanced replication of simian immunodeficiency virus adjacent to catecholaminergic varicosities in primate lymph nodes. J Virol. 2006; 80:4326–4335. [PubMed: 16611891]
- Soneson C, Delorenzi M. A comparison of methods for differential expression analysis of RNA-seq data. BMC Bioinformatics. 2013; 14:91. [PubMed: 23497356]
- Walsh JC, Mandalia S, Gazzard BG. Responses to a 1 month self-report on adherence to antiretroviral therapy are consistent with electronic data and virological treatment outcome. Aids. 2002; 16:269–277. [PubMed: 11807312]

- Wilkins KC, Lang AJ, Norman SB. Synthesis of the psychometric properties of the PTSD checklist (PCL) military, civilian, and specific versions. *Depress Anxiety*. 2011; 28:596–606. [PubMed: 21681864]
- Wilson JM, Kalasinsky KS, Levey AI, Bergeron C, Reiber G, Anthony RM, Schmunk GA, Shannak K, Haycock JW, Kish SJ. Striatal dopamine nerve terminal markers in human, chronic methamphetamine users. *Nat Med*. 1996; 2:699–703. [PubMed: 8640565]

Author Manuscript

Author Manuscript

Author Manuscript

Author Manuscript

Highlights

- Documents stimulant-associated leukocyte gene expression in treated HIV infection
- Elevated tumor necrosis factor – alpha (TNF- α) levels in recent stimulant users
- Stimulant use may contribute to HIV persistence and residual immune dysregulation

Table 1

Demographic, health-related, and psychiatric factors as a function of recent stimulant use

	Stimulant Tox+ (n = 27)	Stimulant Tox- (n = 28)	
	<u>M (SD)</u>	<u>M (SD)</u>	
Age	43.8 (7.2)	45.8 (8.2)	$t(53) = 0.93; p = 0.36$
Time Since HIV Diagnosis (Years)	12.0 (8.3)	15.5 (7.5)	$t(53) = 1.66; p = 0.10$
ART Adherence	77.8 (20.0)	87.8 (21.0)	$t(53) = 1.80; p = 0.08$
CD4+ T-Cell Count (cells/mm³)	642.8 (298.5)	651.5 (286.5)	$t(53) = 0.11; p = 0.91$
Methamphetamine Use Days (Past 30 Days)	10.7 (8.6)	4.0 (6.6)	$t(53) = -3.24; p < 0.01$
ASI Drug Score	0.2 (0.1)	0.2 (0.1)	$t(53) = -0.61; p = 0.55$
ASI Alcohol Score	0.2 (0.1)	0.1 (0.2)	$t(53) = -0.51; p = 0.62$
Depressive Symptom Severity (CES-D)	27.6 (13.7)	21.3 (12.9)	$t(53) = -1.76; p = 0.08$
PTSD Symptom Severity (PCL-C)	49.7 (11.9)	48.4 (15.1)	$t(53) = -0.36; p = 0.72$
Log₁₀ Interleukin-6 (IL-6)	0.4 (0.3)	0.3 (0.3)	$t(53) = -1.66; p = 0.10$
Log₁₀ Tumor Necrosis Factor – Alpha (TNF-α)	1.7 (0.20)	1.6 (0.20)	$t(53) = -2.34; p = 0.02$
	<u>n (%)</u>	<u>n (%)</u>	
Race/Ethnicity			$\chi^2(3) = 3.07; p = 0.38$
Black/African American	1 (4%)	5 (17%)	
White	13 (48%)	13 (46%)	
Hispanic/Latino	10 (37%)	8 (29%)	
Other Ethnic Minority	3 (11%)	2 (7%)	
ART Regimen			$\chi^2(5) = 3.46; p = 0.63$
INSTI & NRTI	7 (26%)	6 (21%)	
Boosted PI & NRTI	9 (33%)	6 (21%)	
NNRTI & NRTI	6 (22%)	11 (39%)	
PI+NNRTI+NRTI	2 (8%)	1 (4%)	
Other HAART	3 (11%)	3 (11%)	
No HAART	0 (0%)	1 (4%)	
ART persistence	11 (41%)	14 (50%)	$\chi^2(1) = 0.48; p = 0.49$
Undetectable HIV Viral Load (< 40 copies/mL)	22 (82%)	24 (86%)	$\chi^2(1) = 0.18; p = 0.67$

Abbreviations: ART = Antiretroviral Therapy; ASI = Addiction Severity Index; CES-D = Center for the Epidemiologic Study - Depression Scale; HAART = Highly Active Anti-Retroviral Therapy; INSTI = Integrase Strand Transfer Inhibitor; M = mean; NNRTI = Non-Nucleoside Reverse Transcriptase Inhibitor; NRTI = Nucleoside Reverse Transcriptase Inhibitor; PCL-C = PTSD Checklist - Civilian Version Score; PI = Protease Inhibitor; PTSD = Post-Traumatic Stress Disorder; SD = Standard Deviation

Table 2

Differentially expressed genes as a function of recent stimulant use.

Entrez Gene ID	Name	Fold Change	Direction	P _{adjusted}	Primary Role(s)
<i>HIV Latency</i>					
29126	CD274	0.79	Up	0.0145	Decreased T-Cell Activation & PD-L1 Binding
8850	KAT2B	0.27	Down	0.0691	Tat Transactivation & Chromatin Remodeling
2212	FCGR2A	0.38	Up	0.0895	HIV Latency (CD32a)
3958	LGALS3	0.52	Down	0.0990	Apoptosis & T-Cell Regulation
8359	HIST1H4A	0.68	Up	0.0990	DNA Transcription
<i>Cell Cycle</i>					
6710	SPTB	0.65	Down	0.0497	Cell Membrane Stability
9764	KIAA0513	0.24	Down	0.0672	Apoptosis & Cytoskeletal Regulation
4303	FOXO4	0.55	Down	0.0681	Insulin Signaling
23039	XPO7	0.36	Down	0.0895	Protein Export from Nucleus
9412	MED21	0.27	Up	0.0990	RNA Transcription
25893	TRIM58	0.59	Down	0.0990	Nuclear Polarization
<i>Immune Activation</i>					
100132417	FCGR1CP	1.17	Up	0.0145	Antibody-Dependent Cytotoxicity
2210	FCGR1B	0.67	Up	0.0691	IFN- γ Signaling
55544	RBM38	0.56	Down	0.0895	Cell Cycle Arrest
2209	FCGR1A	0.62	Up	0.0990	IgG Binding
5609	MAP2K7	0.37	Down	0.0990	Immune Activation & Apoptosis
<i>Cancer</i>					
2195	FAT1	1.31	Down	0.0637	Tumor Suppressor
2530	FUT8	0.29	Up	0.0670	Malignancy & Metastasis
<i>Metabolic</i>					
4122	MAN2A2	0.35	Down	0.0141	Biosynthesis & Metabolism
2548	GAA	0.33	Down	0.0990	Glycolysis
116151	FAM210B	0.56	Down	0.0990	Mitochondrial Activity & Glycolysis
<i>Inflammation</i>					
3340	NDST1	0.30	Down	0.0895	Inflammation

Entrez Gene ID	Name	Fold Change	Direction	P _{adjusted}	Primary Role(s)
<i>Neural/Hormonal</i>					
79098	C1orf116	1.18	Down	0.0145	Androgen-Specific Receptor
11345	GABARAPL2	0.42	Down	0.0145	GABA Receptor Protein
6535	SLC6A8	0.67	Down	0.0335	Creatinine Transport
7504	XK	0.66	Down	0.0336	Amino Acid Transport & Calcium Ion Homeostasis
79056	PRRG4	0.75	Up	0.0342	Calcium Ion Binding
90134	KCNH7	0.55	Up	0.0342	Potassium Voltage-Gated Channel
3759	KCNJ2	0.43	Up	0.0393	Potassium Voltage-Gated Channel
23046	KIF21B	0.19	Down	0.0990	GABA
133	ADM	0.62	Up	0.0990	Hormone Secretion & Angiogenesis
<i>Cardiovascular</i>					
2039	DMTN	0.50	Down	0.0393	Red Blood Cell Structure & Platelet Mobility

Table 3

Perturbed pathways relevant to HIV and neuropsychiatric disorders as a function of recent stimulant use

KEGG ID	Pathway Name	Set Size	P _{adjusted}	Primary Role
<i>HIV Latency</i>				
hsa04110	Cell Cycle	90	< 0.0001	Mitosis
hsa04210	Apoptosis	64	< 0.0001	Programmed Cell Death
hsa04150	mTOR Signaling Pathway	33	0.0969	Metabolism and Survival
hsa00590	Arachidonic Acid Metabolism	32	< 0.0001	Lipid Metabolism
<i>Inflammation</i>				
hsa04060	Cytokine-Cytokine Receptor Interaction	175	< 0.0001	Regulation and Mobilization
hsa04010	MAPK Signaling	163	< 0.0001	Proliferation, Differentiation, and Migration
hsa04062	Chemokine Signaling	127	< 0.0001	Regulation and Mobilization
hsa04630	Jak-STAT Signaling	99	< 0.0001	Antigen Presentation and Inflammation
hsa04664	Fc Epsilon RI Signaling	45	0.0008	Cytokine Gene Transcription
<i>Immune Activation</i>				
hsa04510	Focal Adhesion	136	< 0.0001	Trafficking, Proliferation, and Differentiation of Leukocytes
hsa04514	Cell Adhesion Molecules (CAMs)	84	< 0.0001	Leukocyte Trafficking
hsa04650	Natural Killer Cell Cytotoxicity	81	< 0.0001	Natural Killer Cell Cytotoxicity
hsa04670	Leukocyte Transendothelial Migration	79	0.0011	Leukocyte Trafficking
hsa04660	T-Cell Receptor Signaling	74	< 0.0001	T-Cell Activation
hsa04620	Toll-Like Receptor Signaling	70	< 0.0001	Innate Immune Activation
hsa04666	Fc Gamma R-Mediated Phagocytosis	65	0.0002	Antibody-Dependent Cytotoxicity
hsa04612	Antigen Processing and Presentation	58	< 0.0001	Immune Response
hsa04662	B-Cell Receptor Signaling	49	0.0053	Immune Response
<i>Neuropsychiatric</i>				
hsa04080	Neuroactive Ligand-Receptor Interaction	196	< 0.0001	Neurotransmitters and Neuroendocrine Hormones
hsa05320	Autoimmune Thyroid Disease	43	< 0.0001	Hypothyroidism
hsa00380	Tryptophan Metabolism	28	< 0.0001	Serotonin Synthesis
hsa00350	Tyrosine Metabolism	26	< 0.0001	Catecholamine and Thyroid Hormone Synthesis
<i>Cancer</i>				
hsa05200	Pathways in Cancer	234	< 0.0001	Apoptosis, Focal Adhesion, and Cellular Metabolic Processes