

## Cocaine: A Catalyst for Human Immunodeficiency Virus-Associated Dementia

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**Abstract:** Injection drug use has been recognized as a major risk factor for AIDS from the outset of the epidemic. Cocaine, one of the most widely abused drugs in the United States can both impair the functions of macrophages & CD4<sup>+</sup> lymphocytes and also activate HIV-1 expression in these cells. Cocaine is a multifactorial agent that acts globally to impair the functioning of brain resident cells through multiple pathways. The drug not only promotes virus replication in macrophages, microglia and astrocytes, but can also upregulate CCR5 coreceptor, and reciprocally inhibit its ligands, thereby increasing virus infectivity. Cocaine is known to modulate astroglial function and activation. Cocaine causes a myriad of toxic responses in the neurons: a) it synergizes with viral proteins, Tat and gp120 resulting in exacerbated neuronal apoptosis, b) it causes calcium mobilization and, c) generation of reactive oxygen species. Additionally, cocaine also exerts potent effects on microvascular permeability, thereby impacting the influx of virus-infected inflammatory cells in brain parenchyma. By amplifying the various arms of the toxic responses that characterize HIV-associated dementia (HAD), cocaine skews the balance in favor of the virus leading to accelerated progression and severity of dementia.

**Key words:** Cocaine, HIV, Transcription factors, Blood Brain Barrier.

### INTRODUCTION

Intravenous drug use (IVDU) and HIV infections are two linked global health crises. HIV-1 infection is one of the leading causes of death among Americans 25-44 years of age, and injection drug use accounts for one-third of all new cases of AIDS in the United States. According to the National Household Survey on Drug Abuse it is estimated that in 2004 at least 34.9 million Americans (aged 12 or older) had tried cocaine at least once during their life (SAMSHA, 2004). It has been demonstrated earlier that use of crack cocaine is a risk factor for acquisition of HIV-infection and is also independently associated with progression to AIDS<sup>[1, 2, 3]</sup>. In recent years emergence of a new cohort of HIV-infected individuals that are cocaine-abusers has become evident. It is thus likely that HIV-1 and cocaine interplay in the host might be involved in progression of clinical AIDS.

The CNS is a major target for HIV-1 infection. Within days following infection, HIV-1 enters the CNS where various brain resident cells can serve as reservoirs for HIV-1<sup>[4, 5, 6]</sup>. HIV-associated dementia (HAD) is a clinical dementia syndrome characterized by: a) replication of virus particles in the brain in cells of the macrophage lineage and b) neuronal degeneration. In this syndrome, virus-infected and/or activated macrophages release a barrage of cytokines or inflammatory mediators with neurotoxic properties that ultimately leads to neuronal dysfunction and death<sup>[7, 8, 9, 10]</sup>. The brain is also a target organ for cocaine. Cocaine impairs the functions of macrophages and CD4<sup>+</sup> lymphocytes<sup>[11, 12, 13, 14, 15]</sup> and enhances HIV-1 expression in these cells<sup>[16, 17, 18, 19, 20]</sup>. It has been postulated that cocaine may serve as a co-factor in the susceptibility and progression of HIV-1 infections including HAD<sup>[1, 2, 3]</sup>. Epidemiological studies on drug abusers with AIDS link abuse of cocaine (by different routes), even more than other drugs, to increased

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incidence of HIV seroprevalence and progression to AIDS [21, 22, 23, 24, 25]. Cell culture and murine animal models have provided valuable tools to explore the synergistic interactions of HIV-1 and cocaine in the pathogenesis of HAD. The current review summarizes these studies and the current understanding of these processes.

### **Cocaine-mediated Potentiation of HIV-1 Replication**

***in vitro* and *in vivo*:** Despite the advent of antiretroviral therapy to combat AIDS, cocaine abusers with HIV-1 infection are becoming a newly emerging cohort of HIV-positive individuals. It is therefore critical to understand how the two agents interact to increase the disease severity. Various studies have focused on exploring how cocaine can enhance virus replication in the *in vitro* cell culture systems. Peterson *et al* addressed this question very elegantly using the peripheral blood mononuclear cell (PBMC) coculture system [26, 27]. Briefly, the system comprised of PBMCs from healthy donors incubated in the absence or presence of cocaine prior to activation with a plant lectin, phytohemagglutinin (PHA), and, subsequently these cells were reconstituted with PBMCs that had been infected with a clinical isolate of HIV-1. The authors found that HIV replication, measured by the release of HIV p24 antigen in cell culture fluids was significantly enhanced in activated cells that had been exposed to cocaine [26]. The authors further demonstrated that this effect of cocaine was mediated via the multifunctional cytokine, TGF- $\beta$ . Since immune activation is considered critical for pathogenesis of HIV infection [28, 29, 30], these *in vitro* studies by Peterson *et al* suggest a clinical relevance of cocaine in disease pathogenesis.

Based on the premise that cocaine upregulated virus replication in activated PBMCs, the authors subsequently extended their earlier studies by asking the question whether cocaine could also enhance virus replication in PBMCs that had been activated with CMV, a known enhancer of HIV replication in certain lymphocyte lines [31, 32]. Using the same coculture system as described earlier, PBMCs from CMV positive or CMV-negative donors were pre-treated with cocaine followed by culturing in the presence of HIV-infected PBMCs [27]. These studies demonstrated that while cocaine by itself was not able to trigger HIV-1 replication, in the presence of other activation signals of clinical relevance, such as CMV [31, 33, 34, 35], cocaine was able to synergistically enhance virus replication. Furthermore, similar to the mitogen-stimulated PBMCs,

the mechanism of cocaine-mediated upregulation of virus replication in CMV-stimulated PBMCs occurred via TGF- $\beta$  with a possible involvement of another cytokine, TNF- $\alpha$ . [26, 27, 36].

Additional studies aimed at unraveling the role of cocaine were carried out by Bagasra *et al*, wherein instead of using the coculture system described earlier, which the authors argue may have inherent potential allogenic effects that could confound the data, PBMCs without any stimulation were used to assess the effects of cocaine on modulation of HIV-1 replication. It was found that cocaine-treated unstimulated PBMCs when infected with HIV-1 were also capable of responding with enhanced virus replication as observed by increased HIV-1 p24 antigen levels, syncytium formation and increased viral RNA compared to cells not treated with cocaine [16].

The above findings were focused on mixed cells populations using the PBMC system. Further extension of these studies was carried out in a more relevant cell type, the microglia, which are the resident macrophages of the brain and are the target cells for virus replication in HAD [37]. Microglia play a critical role in defense as well in the neuropathogenic effects of HIV-1. Similar to the effects of cocaine seen in HIV-infected PBMCs, cocaine also enhanced virus replication in microglial cells [16, 18, 19, 27]. Assessment of p24 antigen levels in culture supernatants from the HIV-infected human microglial cells treated with cocaine showed a concentration-dependent increase in viral expression. Extension of these studies using  $\kappa$ -opioid receptor ligands further demonstrated suppression of cocaine-induced potentiation of HIV-1 replication in microglial cells. This effect was mediated by a down-modulation of CCR5, a coreceptor of HIV-1, involving the extracellular signal-regulated kinase1/2 [38]. More recently, it has been found that cocaine-induced HIV-1 expression in these cells also involved the sigma-1 receptors and TGF- $\beta$ 1. This conclusion was arrived at by using the inhibitors specific for sigma-1 receptor and TGF- $\beta$ 1, both of which effectively blocked the cocaine-mediated enhancement of virus replication [39].

Although macrophages and microglial cells are the primary sources of HIV-1 replication in CNS [40, 41], astrocytes are also susceptible to HIV-1 infection, albeit at lower levels [5, 6, 42]. Astrocytes are integral components of the CNS since they maintain a homeostatic environment and actively participate in bidirectional communication with neurons [5, 43, 44]. Following initial infection with HIV-1, astrocytes exhibit a transient surge of viral replication that diminishes to low levels and often persists [5, 6, 42]. It has been estimated that up to twenty percent of astrocytes

can be infected with the virus in HIV-infected patients and remain as reservoirs for latent virus<sup>[6]</sup>. Effect of cocaine on astrocytes in the context of HIV-1 infection has recently been reported by Nair *et al*<sup>[37]</sup>. Since astrocytes make a large proportion of cells in brain and since significant numbers of astrocytes can be infected with HIV-1, and cocaine is known to act as a cofactor in HIV encephalitis (HIV-E), these authors hypothesized that cocaine-induced increases in HIV-1 susceptibility and progression to HIV-E are mediated via the dysregulation of specific proteins in these cells that foster the neuroimmunopathogenesis of HIV-1 infection. The effect of cocaine on HIV-1 infectivity in normal human astrocytes was investigated and it was demonstrated that pre-treatment of astrocytes with cocaine prior to HIV-1 infection significantly upregulated the viral replication as monitored by a significant increase in LTR-R/U5 gene expression<sup>[37]</sup> which represents early stages of reverse transcription of HIV-1. Using the p24 antigen assay, it was demonstrated that culture supernatants from astrocytes treated with cocaine exhibited increased virus replication at day fifteen post-infection. Proteomic analysis by difference gel electrophoresis (DIGE) combined with protein identification through HPLC-MS/MS identified twenty-two proteins in normal human astrocytes that were differentially regulated by cocaine as compared to astrocytes that were not treated with cocaine. Specifically, these proteins comprised the intracellular signaling molecules, translation elongation factor and molecular chaperones<sup>[37]</sup>. These proteins were found to be critical in the neuropathogenesis of HIV-1 infection. These findings have clinical implications for HIV-E since astrocytes make up a significant population of cells in the brain, and their responsiveness to cocaine and/or HIV-1 can lead to increased viral load and subsequent toxicity in the CNS.

Interaction of cocaine and HIV-1 has also been evaluated *in vivo* under more physiologic conditions using a hybrid-mouse model (huPBL SCID mouse) infected with HIV-1 in the presence and absence of cocaine. In this model, systemic cocaine administration led to accelerated HIV-1 infection of human peripheral blood leukocytes (PBL), a decrease in CD4<sup>+</sup> cells and a dramatic rise in circulating virus load<sup>[19]</sup>. HIV infection is known to depress the hypothalamic-pituitary-adrenal axis<sup>[45]</sup>. Cocaine exposure to uninfected huPBL-SCID mice resulted in increased corticosterone production but in concert with HIV-1 infection depressed corticosterone production compared to hu-PBL-SCID mice infected with HIV-1 alone<sup>[46]</sup>. These authors also showed that cocaine induced an upregulation of CCR5 expression on peritoneal cells

from the HIV-infected, cocaine-treated huPBL-SCID mice, which preceded the increase in number of virally infected cells. Using the  $\sigma$ -1 receptor antagonist *Roth et al* demonstrated that cocaine acts via the  $\sigma$ -1 receptor since blocking this receptor abolished the effects of cocaine on HIV-1 replication<sup>[46]</sup>.

In addition to enhancing virus replication in astroglial cells *in vitro*, *in vivo* studies suggested that cocaine administration in mice results in increased proliferation and expression of glial fibrillar acidic protein (GFAP) in the dentate gyrus<sup>[47]</sup>.

**Immunomodulatory Effects of Cocaine in Peripheral Blood Leukocytes:** Cocaine has multiple immunomodulatory effects including the ability to influence cytokine and chemokine release in immunoeffector cells<sup>[22]</sup>. *In vitro* studies using both mouse and human cells have consistently demonstrated that cocaine at physiological concentrations suppresses cytokine release from splenocytes, PBLs and endothelial cells<sup>[12, 48, 49, 50]</sup>. Cocaine has been found to inhibit the IL-2-induced production of IFN- $\gamma$  and IL-8 by PBLs in a dose-responsive manner<sup>[48]</sup>. Cocaine was also shown to decrease IFN- $\gamma$  mRNA expression in PBLs as determined by Northern and slot blot analyses, without affecting the stability of the mRNA. Nuclear run-on assays further demonstrated that cocaine down-regulated the rate of IFN- $\gamma$  transcription<sup>[12]</sup>. Cocaine is also known to modulate the expression of IL-10 a Th2 cytokine that has been shown to promote HIV-1 replication<sup>[51, 52]</sup>.

The response of cocaine on mixed cultures such as PBMCs<sup>[53, 54, 55]</sup> is very different than its response in purified T cells<sup>[11]</sup>. Using purified T cells, Klein *et al* demonstrated that mitogen-stimulated proliferation of these cells was suppressed by cocaine via its down-modulation of calcium mobilization and IL-2 release<sup>[11]</sup>. Interestingly, however, conflicting studies on cocaine-mediated release of IL-2 and calcium release were reported by Matsui *et al*<sup>[56, 57]</sup> when T cells were activated with anti-CD3 antibody. Cocaine can therefore have variable affect on lymphocyte responses depending upon the type of cells and the manner of activation of these cells.

Not only does cocaine have the ability to modulate cytokine expression, it has also been shown to affect the expression of chemokines, which are cytokines with chemoattractant properties. CCLs or  $\beta$ -chemokines play a significant role in resistance to HIV-1 infection and its clinical progression to AIDS. Cocaine has been reported to down-modulate expression of MIP-1 $\beta$  and the  $\beta$ -chemokine receptor, CCR5, a major HIV-1 coreceptor in normal PBMCs<sup>[18]</sup>. Additionally, cocaine also selectively inhibited LPS-induced MIP-1 $\beta$  production by PBMCs isolated from HIV-infected patients<sup>[18]</sup>. Cocaine-mediated decrease in the protective, anti-HIV chemokine, may therefore be one of the mechanisms by which cocaine can accelerate the progression of HIV-1 disease.

**Effects of Cocaine on Tat- and Gp120-Induced Neurotoxicity:**

It is widely accepted that while neurodegeneration is one of the hallmark features of HAD, neurons themselves do not get infected by the virus. Thus it is not the virus, but the viral protein products, Tat and gp120, that can exert neurotoxicity, both *in vitro* and *in vivo* [58, 59, 60, 61, 62, 63, 64]. Furthermore, emerging new *in vitro* data demonstrates that cocaine can amplify the neurotoxic responses of HIV-1 proteins, Tat and gp-120 [60, 65, 66, 67]. Evidence for the interactions of HIV-1 and cocaine in modulating neurotoxicity has been demonstrated in cell culture studies showing enhanced damage and oxidative stress in neurons exposed to Tat and gp120 in the presence of cocaine [68, 69]. Acute exposure of neurons to these viral proteins and cocaine was shown to result in synergistic neurotoxicity mediated, in part, via mitochondrial damage [65].

Using primary rat hippocampal cultures, it has recently been reported that physiologically relevant doses of cocaine can augment Tat-mediated mitochondrial depolarization and intracellular production of reactive oxygen species (ROS) [70]. This subsequently leads to enhanced oxidative stress and neurotoxicity. Additionally, treatment of hippocampal cells with a specific D1 dopamine receptor antagonist blocked the potentiation of Tat toxicity by cocaine [70]. These findings led to the speculation that cocaine enhances Tat-mediated neurotoxicity via modulation of the D1 dopamine receptor-controlled signaling cascades [70].

*In vivo* study by Bagetta *et al* demonstrated that subchronic intraperitoneal administration of cocaine to wild-type Wistar rats in combination with intracerebroventricular injection of recombinant HIV-1 gp-120, results in the enhancement of iNOS expression and apoptosis in neurons in the necocortex region. Cocaine when administered alone was not able to cause neuronal apoptosis. The addition of iNOS inhibitors minimized the neurotoxicity associated with gp120 and cocaine thus suggesting that iNOS plays a critical role in gp120 and cocaine induced neuronal apoptosis.

**Modulation of Transcription Factors by Cocaine:**

It is now widely recognized that oxidative stress can not only induce direct cytotoxic effects [71], but can also activate specific transcription factors that can further upregulate various inflammatory target genes [72]. Redox-regulated transcription factor, AP-1 is considered to be one of the most important oxidative stress-responsive transcription factors involved in cocaine-induced injury in both astroglia and neurons [71, 73]. For example, it has been shown that a single administration of cocaine in the rat brain induces AP-1 DNA-binding activity within the striatum and cerebellum [74]. AP-1 is constituted by a combination of Fos and Jun-related proteins [75]. Several studies have shown that acute administration of cocaine produces a rapid and transient induction in rat brain of c-fos and egr-1 [76, 77, 78] proteins that belong to the family of immediate early genes (IEG). Induction of the

expression of these IEGs by cocaine involves stimulation of dopaminergic [79, 80] and serotonergic receptors in response to cocaine. Since many of the IEGs are known to encode transcription factors, their activation is likely to play a role in the transduction of short-lived environmental signals into long lasting changes in cell function. Thiriet *et al* have further reported that the sodium nitroprusside, a nitrooxide releasing agent and a C-type natriuretic peptide, a neuropeptide, inhibit cocaine-induced IEG expression in rat brain by activating the cGMP signal transduction pathway [81].

Induction of other key transcription factors, NF-KB (key player in HIV-1 replication) [82] and egr-1 (unpublished data) has also shown to be activated by cocaine in HIV-infected monocyte-derived macrophages.

**Cocaine and the Blood Brain Barrier (BBB):**

BBB normally functions as an interface between the blood and brain parenchyma, acting as a watchdog to inhibit the entry of ions, molecules and infiltrating cells into the CNS. During progressive HIV-1 infection, however, there is a breach in this barrier [83, 84, 85, 86] leading to influx of inflammatory cells into the brain resulting in clinical and pathological abnormalities, ranging from mild cognitive impairment to frank dementia. Cocaine, through its direct effect on brain microvascular endothelial cells (BMVECs) and its paracrine effects on BBB via release of pro-inflammatory cytokines, augments HIV-1 neuroinvasion in HAD. Cocaine effects on the enhancement of viral neuroinvasion through the BBB have been studied in great detail [1, 71, 87, 88, 89]. Exposure of brain endothelial cells to cocaine has been demonstrated to up-regulate the expression of endothelial adhesion molecules like intracellular adhesion molecule-1 (ICAM-1), vascular cell adhesion molecule -1 (VCAM-1) and E-selectin and thus increase leukocyte migration across endothelial monolayers [88]. Furthermore, cocaine has been shown to upregulate dendritic cell-specific C type ICAM-3 grabbing nonintegrin (DC-SIGN) and matrix metalloproteinases (MMP) in BMVECs, thereby suggesting that cocaine causes membrane permeability and endothelial transmigration of HIV-infected dendritic cells, which are the first line of defense against HIV-infection [90]. Chronic cocaine treatment has been reported to potentiate chemotactic agent-induced leukocyte-endothelial cell adhesion (LEA) in rats [89]. In addition, cocaine has been reported to decrease cellular glutathione levels, enhance DNA-binding activity of redox-regulated transcription factors (NF-kB and AP-1) and increase expression of TNF- $\alpha$  in human brain endothelial cells, thereby contributing to BBB dysfunction and enhanced leukocyte migration across the cerebral vessel [71]. More recently cocaine has also been shown to remodel BMVEC by up-regulating transcription of genes critical in cytoskeleton organization, signal transduction, cell swelling and vesicular trafficking [91].

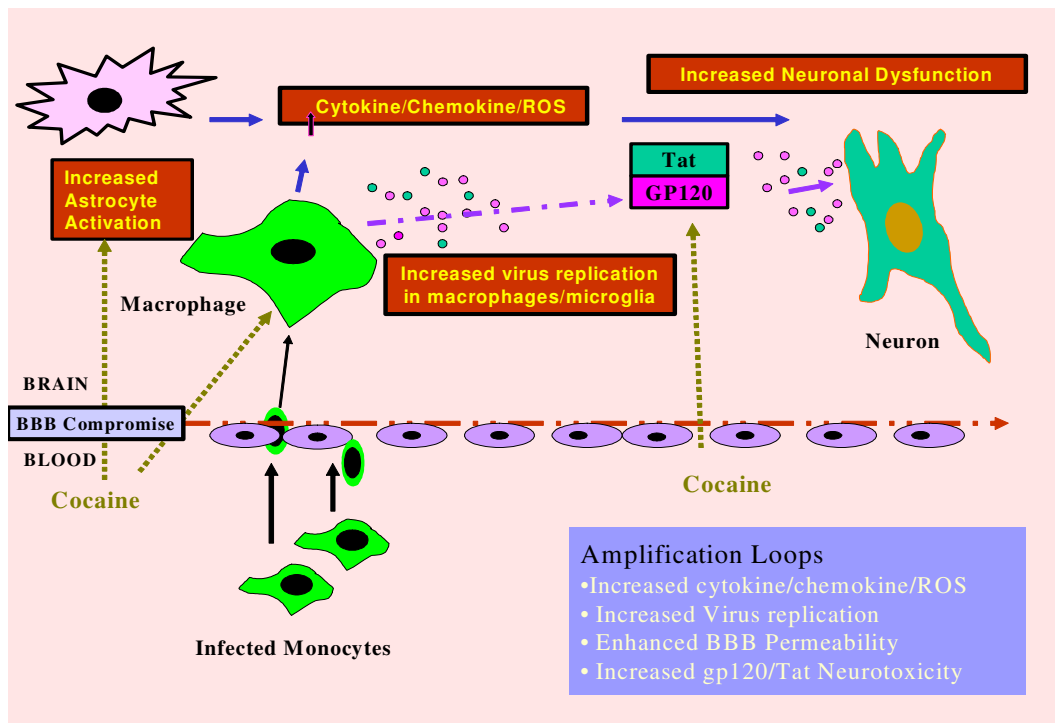


Fig 1: Overall interactive effects of cocaine and HIV-1 in the CNS. Cocaine exposure of macrophages, microglia and astrocytes leads to enhanced virus replication and immunomodulation. Cocaine can also activate astrocyte function and proliferation. Cocaine by its interaction with viral proteins, Tat and gp120, augments neurotoxicity. Additionally, cocaine also exerts potent effects on microvascular permeability, thereby impacting the influx of virus-infected inflammatory cells in brain parenchyma. Thus, cocaine by virtue of its multifactorial properties aids in the progression and severity of HAD.

In summary, cocaine is a multifactorial agent that mediates its effects on several pathways in cells infected with HIV-1 (Figure 1). The drug not only promotes virus replication in PBMCs, macrophages, microglia and astrocytes, but it can also shift the cytokine balance towards a Th2 response via its modulation of IL-10<sup>[51, 52, 92]</sup>. Such a shift has been shown to promote enhancement of CXCR4-utilizing viruses<sup>[93, 94, 95]</sup>. CNS infection with these X4 viruses is becoming increasingly more recognized<sup>[96, 97]</sup>. Additionally, cocaine can upregulate CCR5 coreceptor, and reciprocally inhibit its ligands, thereby increasing virus infectivity. Cocaine is known to modulate astroglial function and activation. Cocaine causes interactive neurotoxicity with viral proteins, Tat and gp120, thereby exacerbating neuronal apoptosis. Additionally, cocaine also exerts potent effects on microvascular permeability, thereby impacting the influx of virus-infected inflammatory cells in brain parenchyma. By amplifying the toxic responses that characterize HAD, cocaine skews the balance in favor

of the virus leading to accelerated progression and severity of disease.

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#### REFERENCES

1. Fiala, M, XH Gan, L Zhang, SD House, T Newton, MC Graves, P Shapshak, M Stins, KS Kim, M Witte, SL Chang, 1998. Cocaine enhances monocyte migration across the blood-brain barrier. Cocaine's connection to AIDS dementia and vasculitis? *Adv.Exp.Med.Biol.*, 437:199-205.
2. Larrat, EP, S Zierler, 1993. Entangled epidemics: cocaine use and HIV disease. *J Psychoactive Drugs*, 25:207-21.
3. Webber, MP, EE Schoenbaum, MN Gourevitch, D Buono, RS Klein, 1999. A prospective study of HIV disease progression in female and male drug users. *AIDS*, 13:257-62.

4. Clarke, JN, JA Lake, CJ Burrell, SL Wesselingh, PR Gorry, P Li, 2006. Novel pathway of human immunodeficiency virus type 1 uptake and release in astrocytes. *Virology*, 348:141-55.
5. Brack-Werner, R, 1999. Astrocytes: HIV cellular reservoirs and important participants in neuropathogenesis. *AIDS*, 13:1-22.
6. Canki, M, JN Thai, W Chao, A Ghorpade, MJ Potash, DJ Volsky, 2001. Highly productive infection with pseudotyped human immunodeficiency virus type 1 (HIV-1) indicates no intracellular restrictions to HIV-1 replication in primary human astrocytes. *J.Virol.*, 75:7925-33.
7. Glass, JD, H Fedor, SL Wesselingh, JC McArthur, 1995. Immunocytochemical quantitation of human immunodeficiency virus in the brain: correlations with dementia. *Ann Neurol JID - 7707449*, 38:755-62.
8. Glass, JD, SL Wesselingh, OA Selnes, JC McArthur, 1993. Clinical-neuropathologic correlation in HIV-associated dementia. *Neurology*, 43:2230-7.
9. Tyor, WR, SL Wesselingh, JW Griffin, JC McArthur, DE Griffin, 1995. Unifying hypothesis for the pathogenesis of HIV-associated dementia complex, vacuolar myelopathy, and sensory neuropathy. *J.Acquir.Immune.Defic.Syndr.Hum.Retrovirol.*, 9:379-88.
10. Gray, F, H Adle-Biasette, F Brion, T Ereau, MI le, V Levy, G Corcket, 2000. Neuronal apoptosis in human immunodeficiency virus infection. *J.Neurovirol.*, 6 Suppl 1:S38-S43.
11. Klein, TW, K Matsui, CA Newton, J Young, RE Widen, H Friedman, 1993. Cocaine suppresses proliferation of phytohemagglutinin-activated human peripheral blood T-cells. *Int.J Immunopharmacol.*, 15:77-86.
12. Mao, JT, M Huang, J Wang, S Sharma, DP Tashkin, SM Dubinett, 1996. Cocaine down-regulates IL-2-induced peripheral blood lymphocyte IL-8 and IFN-gamma production. *Cell Immunol.*, 172:217-23.
13. Baldwin, GC, DP Tashkin, DM Buckley, AN Park, SM Dubinett, MD Roth, 1997. Marijuana and cocaine impair alveolar macrophage function and cytokine production. *Am.J Respir.Crit Care Med.*, 156:1606-13.
14. Eisenstein, TK, ME Hilburger, 1998. Opioid modulation of immune responses: effects on phagocyte and lymphoid cell populations. *J Neuroimmunol.*, 83:36-44.
15. Friedman, H, C Newton, TW Klein, 2003. Microbial infections, immunomodulation, and drugs of abuse. *Clin.Microbiol.Rev.*, 16:209-19.
16. Bagasra, O, RJ Pomerantz, 1993. Human immunodeficiency virus type 1 replication in peripheral blood mononuclear cells in the presence of cocaine. *J Infect.Dis.*, 168:1157-64.
17. Peterson, PK, BM Sharp, G Gekker, PS Portoghese, K Sannerud, HH Balfour, 1990. Morphine promotes the growth of HIV-1 in human peripheral blood mononuclear cell cocultures. *AIDS*, 4:869-73.
18. Nair, MPN, S Mahajan, J Hou, AM Sweet, SA Schwartz, 2000. The stress hormone, cortisol, synergizes with HIV-1 gp-120 to induce apoptosis of normal human peripheral blood mononuclear cells. *Cell Mol.Biol.(Noisy.-le-grand)*, 46:1227-38.
19. Roth, MD, DP Tashkin, R Choi, BD Jamieson, JA Zack, GC Baldwin, 2002. Cocaine enhances human immunodeficiency virus replication in a model of severe combined immunodeficient mice implanted with human peripheral blood leukocytes. *J Infect.Dis.*, 185:701-5.
20. Steele, AD, EE Henderson, TJ Rogers, 2003. Mu-opioid modulation of HIV-1 coreceptor expression and HIV-1 replication. *Virology*, 309:99-107.
21. Anthony, JC, D Vlahov, KE Nelson, S Cohn, J Astemborski, L Solomon, 1991. New evidence on intravenous cocaine use and the risk of infection with human immunodeficiency virus type 1. *Am J Epidemiol.*, 134:1175-89.
22. Baldwin, GC, MD Roth, DP Tashkin, 1998. Acute and chronic effects of cocaine on the immune system and the possible link to AIDS. *J Neuroimmunol.*, 83:133-8.
23. Chaisson, RE, P Bacchetti, D Osmond, B Brodie, MA Sande, AR Moss, 1989. Cocaine use and HIV infection in intravenous drug users in San Francisco. *JAMA*, 261:561-5.
24. Doherty, MC, RS Garfein, E Monterroso, D Brown, D Vlahov, 2000. Correlates of HIV infection among young adult short-term injection drug users. *AIDS*, 14:717-26.
25. Chiasson, MA, RL Stoneburner, DS Hildebrandt, WE Ewing, EE Telzak, HW Jaffe, 1991. Heterosexual transmission of HIV-1 associated with the use of smokable freebase cocaine (crack). *AIDS*, 5:1121-6.
26. Peterson, PK, G Gekker, CC Chao, R Schut, TW Molitor, HH Balfour, 1991. Cocaine potentiates HIV-1 replication in human peripheral blood mononuclear cell cocultures. Involvement of transforming growth factor-beta. *J Immunol.*, 146:81-4.
27. Peterson, PK, G Gekker, CC Chao, R Schut, J Verhoef, CK Edelman, A Erice, HH Balfour, Jr., 1992. Cocaine amplifies HIV-1 replication in cytomegalovirus-stimulated peripheral blood mononuclear cell cocultures. *J Immunol.*, 149:676-80.
28. Rosenberg, ZF, AS Fauci, 1989. Induction of expression of HIV in latently or chronically infected cells. *AIDS Res Hum Retroviruses*, 5:1-4.
29. Levy, JA, 1989. Human immunodeficiency viruses and the pathogenesis of AIDS. *Jama*, 261:2997-3006.
30. Gallo, RC, 1990. Mechanism of disease induction by HIV. *J Acquir Immune Defic Syndr*, 3:380-9.

31. Skolnik, PR, BR Kosloff, MS Hirsch, 1988. Bidirectional interactions between human immunodeficiency virus type 1 and cytomegalovirus. *J Infect Dis*, 157:508-14.
32. Clouse, KA, PB Robbins, B Fernie, JM Ostrove, AS Fauci, 1989. Viral antigen stimulation of the production of human monokines capable of regulating HIV1 expression. *J Immunol*, 143:470-5.
33. Drew, WL, 1988. Cytomegalovirus infection in patients with AIDS. *J Infect Dis*, 158:449-56.
34. Jacobson, MA, J Mills, 1988. Serious cytomegalovirus disease in the acquired immunodeficiency syndrome (AIDS). Clinical findings, diagnosis, and treatment. *Ann Intern Med*, 108:585-94.
35. Schooley, RT, 1990. Cytomegalovirus in the setting of infection with human immunodeficiency virus. *Rev Infect Dis*, 12 Suppl 7:S811-9.
36. Peterson, PK, G Gekker, R Schut, S Hu, HH Balfour, Jr., CC Chao, 1993. Enhancement of HIV-1 replication by opiates and cocaine: the cytokine connection. *Adv.Exp.Med.Biol.*, 335:181-8.
37. Reynolds, JL, SD Mahajan, B Bindukumar, D Sykes, SA Schwartz, MP Nair, 2006. Proteomic analysis of the effects of cocaine on the enhancement of HIV-1 replication in normal human astrocytes (NHA). *Brain Res*, 1123:226-36.
38. Gekker, G, S Hu, MP Wentland, JM Bidlack, JR Lokensgard, PK Peterson, 2004. Kappa-opioid receptor ligands inhibit cocaine-induced HIV-1 expression in microglial cells. *J Pharmacol Exp Ther*, 309:600-6.
39. Gekker, G, S Hu, WS Sheng, RB Rock, JR Lokensgard, PK Peterson, 2006. Cocaine-induced HIV-1 expression in microglia involves sigma-1 receptors and transforming growth factor-beta1. *Int Immunopharmacol*, 6:1029-33.
40. Minagar, A, P Shapshak, R Fujimura, R Ownby, M Heyes, C Eisdorfer, 2002. The role of macrophage/microglia and astrocytes in the pathogenesis of three neurologic disorders: HIV-associated dementia, Alzheimer disease, and multiple sclerosis. *J Neurol Sci*, 202:13-23.
41. Gonzalez-Scarano, F, J Martin-Garcia, 2005. The neuropathogenesis of AIDS. *Nat Rev Immunol*, 5:69-81.
42. Conant, K, C Tornatore, W Atwood, K Meyers, R Traub, EO Major, 1994. In vivo and in vitro infection of the astrocyte by HIV-1. *Adv.Neuroimmunol.*, 4:287-9.
43. Dong, Y, EN Benveniste, 2001. Immune function of astrocytes. *Glia*, 36:180-90.
44. Hansson, E, L Ronnback, 2003. Glial neuronal signaling in the central nervous system. *Faseb J*, 17:341-8.
45. Kumar, M, AM Kumar, D Waldrop, MH Antoni, N Schneiderman, C Eisdorfer, 2002. The HPA axis in HIV-1 infection. *J Acquir Immune Defic Syndr*, 31 Suppl 2:S89-93.
46. Roth, MD, KM Whittaker, R Choi, DP Tashkin, GC Baldwin, 2005. Cocaine and sigma-1 receptors modulate HIV infection, chemokine receptors, and the HPA axis in the huPBL-SCID model. *J.Leukoc.Biol.*, 78:1198-203.
47. Fattore, L, MC Puddu, S Picciau, A Cappai, W Fratta, GP Serra, S Spiga, 2002. Astroglial in vivo response to cocaine in mouse dentate gyrus: a quantitative and qualitative analysis by confocal microscopy. *Neuroscience*, 110:1-6.
48. Mao, JT, LX Zhu, S Sharma, K Chen, M Huang, SJ Santiago, J Gulsurd, DP Tashkin, SM Dubinett, 1997. Cocaine inhibits human endothelial cell IL-8 production: the role of transforming growth factor-beta. *Cell Immunol*, 181:38-43.
49. Wang, Y, DS Huang, RR Watson, 1994. In vivo and in vitro cocaine modulation on production of cytokines in C57BL/6 mice. *Life Sci.*, 54:401-11.
50. Watzl, B, G Chen, P Scuderi, S Pirozhkov, RR Watson, 1992. Cocaine-induced suppression of interferon-gamma secretion in leukocytes from young and old C57BL/6 mice. *Int J Immunopharmacol*, 14:1125-31.
51. Stanulis, ED, SD Jordan, JA Rosecrans, MP Holsapple, 1997. Disruption of Th1/Th2 cytokine balance by cocaine is mediated by corticosterone. *Immunopharmacology*, 37:25-33.
52. Gardner, B, LX Zhu, MD Roth, DP Tashkin, SM Dubinett, S Sharma, 2004. Cocaine modulates cytokine and enhances tumor growth through sigma receptors. *J Neuroimmunol.*, 147:95-8.
53. Berkeley, MB, S Daussin, MC Hernandez, BM Bayer, 1994. In vitro effects of cocaine, lidocaine and monoamine uptake inhibitors on lymphocyte proliferative responses. *Immunopharmacol Immunotoxicol*, 16:165-78.
54. Delafuente, JC, CL DeVane, 1991. Immunologic effects of cocaine and related alkaloids. *Immunopharmacol Immunotoxicol*, 13:11-23.
55. Klein, TW, CA Newton, H Friedman, 1988. Suppression of human and mouse lymphocyte proliferation by cocaine. *Adv Biochem Psychopharmacol*, 44:139-43.
56. Matsui, K, H Friedman, TW Klein, 1993. Molecular mechanisms associated with cocaine-induced modulation of human T lymphocytes proliferation. *Adv Exp Med Biol*, 335:127-34.
57. Matsui, K, H Friedman, TW Klein, 1992. Cocaine augments proliferation of human peripheral blood T-lymphocytes activated with anti-CD3 antibody. *Int J Immunopharmacol*, 14:1213-20.
58. New, DR, M Ma, LG Epstein, A Nath, HA Gelbard, 1997. Human immunodeficiency virus type 1 Tat protein induces death by apoptosis in primary human neuron cultures. *J.Neurovirol.*, 3:168-73.
59. Bansal, AK, CF Mactutus, A Nath, W Maragos, KF Hauser, RM Booze, 2000. Neurotoxicity of HIV-1 proteins gp120 and Tat in the rat striatum. *Brain Res.*, 879:42-9.

60. Gurwell, JA, A Nath, Q Sun, J Zhang, KM Martin, Y Chen, KF Hauser, 2001. Synergistic neurotoxicity of opioids and human immunodeficiency virus-1 Tat protein in striatal neurons in vitro. *Neuroscience*, 102:555-63.
61. Savio, T, G Levi, 1993. Neurotoxicity of HIV coat protein gp120, NMDA receptors, and protein kinase C: a study with rat cerebellar granule cell cultures. *J.Neurosci.Res.*, 34:265-72.
62. Lipton, SA, NJ Sucher, PK Kaiser, EB Dreyer, 1991. Synergistic effects of HIV coat protein and NMDA receptor-mediated neurotoxicity. *Neuron*, 7:111-8.
63. Kaul, M, GA Garden, SA Lipton, 2001. Pathways to neuronal injury and apoptosis in HIV-associated dementia. *Nature*, 410:988-94.
64. Kaul, M, SA Lipton, 1999. Chemokines and activated macrophages in HIV gp120-induced neuronal apoptosis. *Proc.Natl.Acad.Sci.U.S.A.*, 96:8212-6.
65. Turchan, J, C Anderson, KF Hauser, Q Sun, J Zhang, Y Liu, PM Wise, I Kruman, W Maragos, MP Mattson, R Booze, A Nath, 2001. Estrogen protects against the synergistic toxicity by HIV proteins, methamphetamine and cocaine. *BMC.Neurosci.*, 2:3.
66. Nath, A, KF Hauser, V Wojna, RM Booze, W Maragos, M Prendergast, W Cass, JT Turchan, 2002. Molecular basis for interactions of HIV and drugs of abuse. *J Acquir.Immune Defic.Syindr.*, 31 Suppl 2:S62-S9.
67. Maragos, WF, KL Young, JT Turchan, M Guseva, JR Pauly, A Nath, WA Cass, 2002. Human immunodeficiency virus-1 Tat protein and methamphetamine interact synergistically to impair striatal dopaminergic function. *J Neurochem*, 83:955-63.
68. Nath, A, C Anderson, M Jones, W Maragos, R Booze, C Mactutus, J Bell, KF Hauser, M Mattson, 2000. Neurotoxicity and dysfunction of dopaminergic systems associated with AIDS dementia. *J Psychopharmacol.*, 14:222-7.
69. Koutsilieris, E, ME Gotz, S Sopper, U Sauer, M Demuth, V ter Meulen, P Riederer, 1997. Regulation of glutathione and cell toxicity following exposure to neurotropic substances and human immunodeficiency virus-1 in vitro. *J Neurovirol*, 3:342-9.
70. Aksenov, MY, MV Aksenova, A Nath, PD Ray, CF Mactutus, RM Booze, 2006. Cocaine-mediated enhancement of Tat toxicity in rat hippocampal cell cultures: the role of oxidative stress and D1 dopamine receptor. *Neurotoxicology*, 27:217-28.
71. Lee, YW, B Hennig, M Fiala, KS Kim, M Toborek, 2001. Cocaine activates redox-regulated transcription factors and induces TNF-alpha expression in human brain endothelial cells. *Brain Res.*, 920:125-33.
72. Schreck, R, K Albermann, PA Baeuerle, 1992. Nuclear factor kappa B: an oxidative stress-responsive transcription factor of eukaryotic cells (a review). *Free Radic Res Commun*, 17:221-37.
73. Malaplate-Armand, C, P Becuwe, L Ferrari, C Masson, M Dauca, S Visvikis, H Lambert, AM Batt, 2005. Effect of acute and chronic psychostimulant drugs on redox status, AP-1 activation and pro-enkephalin mRNA in the human astrocyte-like U373 MG cells. *Neuropharmacology*, 48:673-84.
74. Couceyro, P, KM Pollock, K Drews, J Douglass, 1994. Cocaine differentially regulates activator protein-1 mRNA levels and DNA-binding complexes in the rat striatum and cerebellum. *Mol Pharmacol*, 46:667-76.
75. Pennypacker, K, 1998. AP-1 transcription factors: short- and long-term modulators of gene expression in the brain. *Int Rev Neurobiol*, 42:169-97.
76. Hope, B, B Kosofsky, SE Hyman, EJ Nestler, 1992. Regulation of immediate early gene expression and AP-1 binding in the rat nucleus accumbens by chronic cocaine. *Proc.Natl.Acad.Sci.U.S.A.*, 89:5764-8.
77. Bhat, RV, JM Baraban, 1993. Activation of transcription factor genes in striatum by cocaine: role of both serotonin and dopamine systems. *J.Pharmacol.Exp.Ther.*, 267:496-505.
78. Moratalla, R, HA Robertson, AM Graybiel, 1992. Dynamic regulation of NGFI-A (zif268, egr1) gene expression in the striatum. *J.Neurosci.*, 12:2609-22.
79. Drago, J, CR Gerfen, H Westphal, H Steiner, 1996. D1 dopamine receptor-deficient mouse: cocaine-induced regulation of immediate-early gene and substance P expression in the striatum. *Neuroscience*, 74:813-23.
80. Mailleux, P, F Zhang, JJ Vanderhaeghen, 1992. The dopamine D1 receptor antagonist SCH-23390 decreases the mRNA levels of the transcription factor zif268 (krox-24) in adult rat intact striatum--an in situ hybridization study. *Neurosci Lett*, 147:182-4.
81. Thiriet, N, D Aunis, J Zwiller, 2002. The nitric oxide releasing agent sodium nitroprusside modulates cocaine-induced immediate early gene expression in rat brain. *Ann N Y Acad Sci*, 965:47-54.
82. Dhillon, N, R. Williams, F. Peng *et al.*, 2007. Cocaine-mediated Enhancement of Virus Replication in Macrophages: Implications for HIV-Dementia. *J Neurovirology*, In press.
83. Burger, DM, CA Boucher, PL Meenhorst, CL Kraayeveld, P Portegies, JW Mulder, RM Hoetelmans, JH Beijnen, 1997. HIV-1 RNA levels in the cerebrospinal fluid may increase owing to damage to the blood-brain barrier. *Antivir Ther*, 2:113-7.



84. Nottet, HS, Y Persidsky, VG Sasseville, AN Nukuna, P Bock, QH Zhai, LR Sharer, RD McComb, S Swindells, C Soderland, HE Gendelman, 1996. Mechanisms for the transendothelial migration of HIV-1-infected monocytes into brain. *J Immunol*, 156:1284-95.
85. Dallasta, LM, LA Pisarov, JE Esplen, JV Werley, AV Moses, JA Nelson, CL Achim, 1999. Blood-brain barrier tight junction disruption in human immunodeficiency virus-1 encephalitis. *Am J Pathol*, 155:1915-27.
86. Avison, MJ, A Nath, R Greene-Avison, FA Schmitt, RA Bales, A Ethisham, RN Greenberg, JR Berger, 2004. Inflammatory changes and breakdown of microvascular integrity in early human immunodeficiency virus dementia. *J Neurovirol*, 10:223-32.
87. Fiala, M, C Gujuluva, O Berger, M Bukrinsky, KS Kim, MC Graves, 2001. Chemokine receptors on brain endothelia--keys to HIV-1 neuroinvasion? *Adv.Exp.Med.Biol.*, 493:35-40.
88. Gan, X, L Zhang, O Berger, MF Stins, D Way, DD Taub, SL Chang, KS Kim, SD House, M Weinand, M Witte, MC Graves, M Fiala, 1999. Cocaine enhances brain endothelial adhesion molecules and leukocyte migration. *Clin.Immunol.*, 91:68-76.
89. Chang, SL, J Bersig, B Felix, M Fiala, SD House, 2000. Chronic cocaine alters hemodynamics and leukocyte-endothelial interactions in rat mesenteric venules. *Life Sci.*, 66:2357-69.
90. Nair, MP, SD Mahajan, SA Schwartz, J Reynolds, R Whitney, Z Bernstein, RP Chawda, D Sykes, R Hewitt, CB Hsiao, 2005. Cocaine modulates dendritic cell-specific C type intercellular adhesion molecule-3-grabbing nonintegrin expression by dendritic cells in HIV-1 patients. *J Immunol*, 174:6617-26.
91. Fiala, M, AJ Eshleman, J Cashman, J Lin, AS Lossinsky, V Suarez, W Yang, J Zhang, W Popik, E Singer, F Chiappelli, E Carro, M Weinand, M Witte, J Arthos, 2005. Cocaine increases human immunodeficiency virus type 1 neuroinvasion through remodeling brain microvascular endothelial cells. *J Neurovirol.*, 11:281-91.
92. Dhillon, N, R. Williams, F. Peng, Yi-jou Tsai, S. Dhillon, B. Nicolay, M. Gadgil, A. Kumar and S. Buch., In press. Cocaine-mediated Enhancement of Virus Replication in Macrophages: Implications for HIV-Dementia. *J Neurovirology*.
93. Buch, S, D Pinson, CL King, R Raghavan, Y Hou, Z Li, I Adany, A Hicks, F Villinger, A Kumar, O Narayan, 2001. Inhibitory and enhancing effects of IFN-gamma and IL-4 on SHIV(KU) replication in rhesus macaque macrophages: correlation between Th2 cytokines and productive infection in tissue macrophages during late-stage infection. *Cytokine JID - 9005353*, 13:295-304.
94. Buch, SJ, F Villinger, D Pinson, Y Hou, I Adany, Z Li, R Dalal, R Raghavan, A Kumar, O Narayan, 2002. Innate differences between simian-human immunodeficiency virus (SHIV)(KU-2)-infected rhesus and pig-tailed macaques in development of neurological disease. *Virology JID - 0110674*, 295:54-62.
95. Dhillon, NK, Y Sui, R Potula, S Dhillon, I Adany, Z Li, F Villinger, D Pinson, O Narayan, S Buch, 2005. Inhibition of pathogenic SHIV replication in macaques treated with antisense DNA of interleukin-4. *Blood*, 105:3094-9.
96. Hicks, A, R Potula, YJ Sui, F Villinger, D Pinson, I Adany, Z Li, C Long, P Cheney, J Marcario, F Novembre, N Mueller, A Kumar, E Major, O Narayan, S Buch, 2002. Neuropathogenesis of lentiviral infection in macaques: roles of CXCR4 and CCR5 viruses and interleukin-4 in enhancing monocyte chemoattractant protein-1 production in macrophages. *Am.J.Pathol.*, 161:813-22.
97. Yi, Y, W Chen, I Frank, J Cutilli, A Singh, L Starr-Spires, J Sulcove, DL Kolson, RG Collman, 2003. An unusual syncytia-inducing human immunodeficiency virus type 1 primary isolate from the central nervous system that is restricted to CXCR4, replicates efficiently in macrophages, and induces neuronal apoptosis. *J Neurovirol.*, 9:432-41.