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[1]CIDMTR, Dept. of Medicine, University of Minnesota Medical School, Minneapolis, MN 55455-0000.

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Stempel School of Public Health, Miami, FL 33199-0000, [4]Universidad Central del Caribe, School of Medicine, Puerto Rico, 00960, [5]University of Miami, School of Nursing and Health Studies, Miami, FL 33124-0000.

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[1]Department of Immunology, College of Medicine, Miami, FL 33155-0000.

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T-35 **DIFFERENTIAL INHIBITORY EFFECTS OF HIV-1 TAT PROTEIN ON [3H]DOPAMINE UPTAKE AND [3H]WIN 35,428 BINDING SITES IN RAT STRIATAL SYNAPTOSOMES**. J Zhu[1], CF Mactutus[1], RM Booze[1]; [1]Univ. of South Carolina, Dept. of Psychology, Columbia, SC 29208-0000.

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SS-1 **Do opiates increase the risk of sepsis? A clinical perspective**. A Brack[1], HL Rittner[1]; [1]Klinik für Anesthesiology und Intensivmedizin, Charité - Universitätsmedizin Berlin, Berlin, 12200

SS-2 **ETHANOL MODULATION OF ASTROGLIAL CHEMOKINE EXPRESSION: IMPLICATIONS IN NEUROAIDS**. RL Davis[1]; [1]Department of Pharmacology/Physiology, Oklahoma State University Ctr for Health Sciences, Tulsa, OK 74107-0000.

SS-3 **A NEW ANTIOXIDANT PREVENTS TOXICITY OF HIV PROTEINS WITH METHAMPHETAMINE.** N Ercal[1], W Banks[2], L Abraham[1], T Otamis price[2], X Zhang[1]; [1]Biochemistry, Missouri University of Science & Technology, Rolla, MO 65409-0000, [2]School of Medicine, Saint Louis University, St. Louis, MO 63106-0000.

SS-4 **NEW DIRECTIONS AND INITIATIVES AT NIDA**. L Erinoff[1]; [1]AIDS Research Program, National Institute on Drug Abuse, Bethesda, MD 20892-0000.

SS-5 **NOVEL INDUSTRIAL-ACADEMIC PARTNERSHIPS IN DEVELOPING CELL-BASED NANOFROMULATIONS FOR NEURODEGENERATIVE DISEASES**. HE Gendelman[1], BE Rabinow[2]; [1]Department of Pharmacology and Exp. Neuroscience, University of Nebraska Medical Center, Omaha, NE 68198-5880, Baxter Healthcare Corp., Round Lake, IL 60073-0000.

SS-6 **SPHINGOLIPID, REDOX AND INFLAMMATORY PERSPECTIVES ON NEURODEGENERATION IN AIDS**. NJ Haughey[1]; [1]Neuroimmunology and Neurological Infections, The Johns Hopkins University School of Medicine, Baltimore, MD 21287-0000.

SS-7 **CANNABINOID SUPPRESSION OF THE T HELPER CELL POLARIZING FUNCTION OF DENDRITIC CELLS**. T Klein[1], C Newton[1], L Lu[2]; [1]Molecular Medicine, College of Medicine, University of South Florida, Tampa, FL 33612-0000, [2]Cellular Immunology Section, NIH, NIAID, Bethesda, MD 20892-0000.

SS-8 **ALCOHOL AND NEUROAIDS: NEUROIMAGING AND COGNITION**. DJ Meyerhoff[1]; [1]Center for Imaging of Neurodegenerative Diseases, VA Medical Center and University of California, San Francisco, CA 94121-0000.

SS-9 **HIV-1 CNS INFECTION: BLOOD BRAIN BARRIER (BBB) INJURY AND CO-MORBIDITY FACTORS**. Y Persidsky[1]; [1]Path/Microbiol & Pharmacol/Experimental Neurosci, University of Nebraska Medical Center, Omaha, NE 68198-5215.

SS-10 **SIGNALING PATHWAYS IN HIV-1 TAT-INDUCED CEREBROVASCULAR PATHOLOGY**. M Toborek[1]; [1]Department of Neurosurgery, University of Kentucky Medical Center, Lexington, KY

SS-11 **MODULATION OF CANNABINOID RECEPTOR ACTIVATION AS A NEUROPROTECTIVE STRATEGY**. RF Tuma[1], M Zhang[1], BR Martin[2], RK Razdan[3], MW Adler[1], D Ganea[1]; [1]Center for Substance Abuse Research, Temple University School of Medicine, Philadelphia, PA 19140-0000, [2]Department of Pharmacology and Toxicology,, Virginia Commonwealth University School of Medicin, Richmond, VA 23298-0000, [3]Research and Developmet, Organix Inc., Woburn, MA 01811-0000.40536-0000.

SS-12 **CHRONIC COCAINE ADMINISTRATION TO SCID MICE WITH HIV ENCEPHALITIS**. WR Tyor[2], WC Griffin[2], LD Middaugh[2]; [1]Department of Neurosciences, Medical University of South Carolina, Charleston, SC 29425-0000, [2]Department of Psychiatry, Medical University of South Carolina, Charleston,SC 29425-0000.

POSTER SESSION 1

Young Investigators Poster Session (Abstracts)

W-1 **EFFECTS OF CHRONIC ETHANOL AND ETHANOL WITHDRAWAL ON PROINFLAMMATORY INDUCED CXCL10 EXPRESSION IN HUMAN ASTROGLIA**. D Armstrong[1], D Buck[1], A Nath[2], RL Davis[1]; [1]Department of Physiology, OSU-CHS, Tulsa, OK 74107, [2]Department of Neurology, Johns Hopkins University, Baltimore, MD 21287.

Alcohol abuse is prevalent among HIV-1 infected individuals, and increasing evidence suggests that alcohol exacerbates HIV-1 neuropathogenesis. The chemokine CXCL10 is elevated in HIV infection, but the neurotoxic and neuroprotective contributions of CXCL10 remain to be fully elucidated. We have characterized the in vitro effects of ethanol and ethanol withdrawal on human astroglial CXCL10 expression. Chronic ethanol (3 d) had minimal effect on TNFalpha induced CXCL10 expression, but chronic ethanol followed by 24 h withdrawal enhanced TNFalpha induced CXCL10 expression. Conversely, chronic ethanol, and to a greater degree ethanol withdrawal, reduced IFNgamma induced CXCL10 expression. To mimic HIV-1 associated neuroinflammation, HIV-1 Tat1-72 protein was included as a proinflammatory agent. Initial findings suggest Tat1-72 alone failed to induce CXCL10 expression, but Tat1-72 potentiated IFNgamma induced CXCL10 expression. IFNgamma + Tat1-72 induced CXCL10 expression was inhibited by chronic ethanol and more so by ethanol withdrawal. Subsequent studies are needed to better understand the impact of HIV-1 and ethanol interactions on neuroinflammation. Supported by NIH AA014955 (RLD)

W-2 **HETEROLOGOUS SENSITIZATION OF CAMP SIGNALING ENHANCES HIV-1 GENE EXPRESSION IN TF-1 BONE MARROW PROGENITOR CELLS THROUGH THE PKA/CREB SIGNALING PATHWAY**. A Banerjee[1], A Alexaki[1], B Wigdahl[1], MR Nonnemacher[1]; [1]Dept. of Microbiology and Immunology, Drexel University College of Medicine, Philadelphia, PA 19102.

Chronic opioid exposure has been shown to adversely affect the innate and adaptive immune system. Additionally, the deleterious effects of mu-opioids on selected hematopoietic cell subtypes is well documented. However, the molecular mechanisms of such alterations have yet to be fully defined. The human CD34+/CD38+ TF-1 bone marrow progenitor cell line has been developed as an in-vitro model for studying chronic opioid-induced effects on progenitor cell development, studying the signaling mechanism involved, and analyzing how the downstream molecular events modulate HIV-1 gene expression in these cells. We have identified functional mu opioid receptor isoform-1 (MOR-1) on the surface of these cells and propose to elucidate the effects of chronic opioid exposure on HIV-1 promoter activation. In this regard, we have confirmed that increased adenylate cyclase activation as a result of prolonged mu opioid receptor stimulation results in increased cyclic AMP (cAMP) accumulation in progenitor cells that serves as a molecular trigger in inducing multiple downstream signaling events. To this end, the experiments have revealed the importance of the Protein Kinase A pathway and the associated target transcription factors in increased HIV-1 promoter binding as well as LTR-directed transcription. Studies are also underway to investigate the effects of the cAMP pathway in regulating bone marrow progenitor cell proliferation and survival. Supported by NIDA/R01 DA19807-03

W-3 **IMMUNOSUPPRESSIVE EFFECT OF MORPHINE ON ACINETOBACTER BAUMANNII INFECTION**. JM Breslow[1], JJ Meissler[1], PB Spence[1], JP Gaughan[1], MW Adler[1], MA Monroy[1], TK Eisenstein[1]; [1]Center for Substance Abuse Research, Temple University School of Medicine, Philadelphia, PA 19140-0000.

Multiply drug-resistant Acinetobacter baumannii are usually nosocomial pathogens causing infection in immunosuppressed patients in intensive care units. Infections among healthy, but wounded, military personnel in Iraq have caused concern. We proposed that morphine, administered for analgesia on the battlefield, is immunosuppressive and predisposes to A. baumannii infection. To test this hypothesis, an intraperitoneal (i.p.) infection model was established in 2 strains of mice, examining male and female animals using a strain of A. baumannii obtained from Walter Reed Army Institute of Research. Morphine administered continuously for 48 hr by subcutaneous implantation of a slow-release morphine pellet resulted in 100% mortality

of animals challenged with a 0.1 LD50 dose of A. baumannii, whereas all animals receiving placebo pellets survived. The lethal effect could be blocked by administering the opioid receptor antagonist, naltrexone. Acinetobacter burdens in the blood, spleens, livers, and lungs of morphine-treated mice, assayed 12h after i.p. challenge, were significantly higher than those in placebo-treated mice, confirming that mortality was due to potentiated growth of the bacteria. No effect of morphine on growth or viability of A. baumannii was observed over a 10^6-fold dose range when added to cultures in vitro. These results support the hypothesis that morphine potentiates A. baumanii infection in mice. Supported by W81XWH-06-1-0147

W-4 **EXPRESSION AND FUNCTION OF THE CHEMOKINE RECEPTOR CXCR4 IN THE BRAIN OF MORPHINE-TREATED ANIMALS**. S Burbassi[1], TH Peiris[1], SH Chang[2], KJ Simansky[1], O Meucci[1]; [1]Department of Pharmacology and Physiology, Drexel University College of Medicine, Philadelphia, PA 19102-0000, [2]Institute of NeuroImmune Pharmacology, College of Arts and Science Seton Hall University, South Orange, NJ 07079-0000.

The chemokine CXCL12 and its receptor CXCR4 have been increasingly studied in the CNS due to their critical roles in neuronal patterning and survival. CXCR4 is also one of the major HIV-1 co-receptors and is involved in HIV neuropathogenesis. Recent evidence suggests that drugs of abuse, including opiates, can facilitate progression to neuroAIDS. Here we studied the effect of in vivo morphine treatment on CXCR4 function and expression in control rats, HIVTg rats, and MOR-deficient mice. The [35S]GTPgS incorporation assay was used to determine CXCL12-induced stimulation of GTP □binding□ in various brain areas, while RT-PCR and Western blot were used to evaluate changing in CXCR4 mRNA or protein level. Young animals are treated with Morphine (with or without mu-receptor antagonists pretreatment) for short (1-24 hrs), relatively long (3-5 days) and chronic (7 days) time periods. Pups were then sacrificed at different times. The data show a reduction in CXCR4 coupling to G proteins after morphine pre-treatment in several brain areas, including cortex and hippocampus. However, CXCR4 mRNA was not significantly affected by morphine. Similar results were obtained in HIVTg rats treated with morphine pellets, though CXCR4 mRNA levels appear to be generally reduced in the cortex of these animals as compared to the wild type controls. Ongoing experiments are evaluating protein levels of CXCR4 and testing CXCL12-induced responses in MOR-deficient mice. Thus, impairment of CXCR4 by opiates may contribute to neurological deficits associated with HIV and other neuroinflammatory conditions. Supported by NIH grants DA15014 and DA19808 to OM and DK67648 to KJS

W-5 **TRANSCRIPTIONAL REGULATION OF THE MAJOR HIV CO-RECEPTORS CCR5 & CXCR4 BY THE KAPPA OPIOID RECEPTOR (KOR).** M.J. Finley, T.J. Rogers; Center for Substance Abuse Research, Temple University School of Medicine, Philadelphia, PA 19140.

We report here that activation of KOR down-regulates transcription of CCR5 & CXCR4. Transcription factor array analysis revealed a possible involvement of the interferon (IFN) regulatory pathway in this process. Increased mRNA production of interferon regulatory factor (IRF) 1 & 2 and IFN-gamma have been detected by real-time PCR. Using a bioinformatic approach to search for potential transcription elements, IRF and IFN-gamma elements have been identified in both the CCR5 & CXCR4 promoters. Studies using primary human monocytes have revealed decreased expression of CCR5 and CXCR4 following IFN-gamma treatment. Reporter assays are underway to elucidate promoter element(s) responsible for decreased transcription of CCR5 & CXCR4. Characterization of this novel pathway linking opioid and interferon signaling pathways will have several benefits, including the potential use of KOR agonists as therapeutic tools for the treatment of HIV-1 infection or certain inflammatory diseases. (Supported by NIH grants: DA14230, DA16544, & P30 DA13429)

W-6 **IL-1BETA-MEDIATED REGULATION OF ASTROCYTE TISSUE INHIBITOR OF METALLOPROTEINASES-1 (TIMP-1): NEUROPROTECTIVE MECHANISMS BEYOND REGULATION OF MMP ACTIVITIES**. CY Chao[1], K Borgmann[1], S Holter[1], R Persidsky[1], L Wu[1], A Ghorpade[1]; [1]Pharmacology and Experimental Neuroscience, University of Nebraska Medical Center, Omaha, NE 68198-0000.

Astrocytes acutely exposed to proinflammatory cytokines such as IL-1beta upregulate TIMP-1 (Suryadevara et al., 2003; Crocker et al., 2006a; Gardner et al., 2006). Since the TIMPs have exhibited diverse non-classical effects, the induction of TIMP-1 in neuroinflammation likely serves multiple functions in addition to modulating MMP activity. Our studies examined IL-1beta immune activation and siRNA knock-down of TIMP-1 in astrocytes, and subsequent neurotoxic or neuroprotective potentials of the astrocyte-conditioned media. Further, we used staurosporine, a broad-spectrum kinase inhibitor, macrophage-tropic HIV-1 virions, and the excitotoxic neurotransmitter glutamate to induce cell death in cultured primary human neurons. Simultaneous treatment with TIMP-1 for 24h had similar neuroprotective effects as treatment using brain-derived neurotrophic factor (BDNF), a classical neurotrophin with neuroprotective activities. Moreover, such protective effects were also seen with TIMP-1-T2G (Thr2 mutated to Gly), an engineered mutant with no MMP binding or inhibition (Meng et al., 1999). Treatment with TIMP-2 or TIMP-3 as controls did not provide consistent protective effects. This indicates that TIMP-1 neuroprotective mechanisms may be independent on MMPs. Neurotrophic signaling by TIMP-1 thus emerges as a possible pathway. Preliminary data suggests that TIMP-1 upregulates the anti-apoptotic Bcl-2.

This is an exciting potential addition to the growing list of non-classical functions of TIMP-1 in the central nervous system. Supported by NINDS/RO1 NS48837-01A1

W-7 **HIV NEF PROMOTES THE ACTIVATION OF CAMKII IN PRIMARY ASTROCYTES**. G Chompre[1], J Porter[1], RJ Noel jr.[1]; [1]Biochemistry Department, Ponce School of Medicine, Ponce , PR 00716-0000.

Even after the introduction of HAART, motor and cognitive impairments persist in HIV infected persons. Non-productive infection in astrocytes, through the synthesis of viral proteins including Nef, contributes to this neuropathology. Nef alters cellular signaling pathways through interactions that may depend on myristoylation of its N-terminus. One of these interactions is between camodulin (CAM) and Nef promoting the releasing of cytokines including MCP-1. The other proteins involved in this signaling pathway have not been specifically identified. Our hypothesis is that the interaction between CAM and myristoylated HIV-1 Nef produce phosphorylation in calcium calmodulin kinase II (CaMKII) that results in the production of proinflammatory molecules by astrocytes. To achieve this hypothesis we used transfection to deliver Nef to astrocytes and measured changes in cytokine production. We also found an increase in phosphorylated CaMKII. Calcium channel and CaMKII inhibitors modify these responses. Finally, we tested the ability of astrocytes treated with Nef to affect neuron morphology/function using organotypic hippocampal slice co-culture with treated astrocytes. This work provides support that Nef signaling through CaM/CaMKII in astrocytes can contribute to inflammation and damage in HIV neuropathology. Supported by NIGMS 008239,NCRR 003050

W-8 **METHAMPHETAMINE-INDUCED NEUROTOXICITY: POSSIBLE ROLES FOR MATRIX METALLOPROTEINASES**. NF Fazo[1], BK Yamamoto[1]; [1]Laboratory of Neurochemistry, Dept of Pharmacology, Boston University School of Medicine, Boston, MA 02118-0000.

Methamphetamine (METH) is a widely abused psychostimulant that increases the release of dopamine (DA), serotonin (5-HT), and glutamate (Glu). High doses of METH produce long-term neurodegeneration that is mediated by free radicals, mitochondrial dysfunction, and excitotoxicity. More recently, inflammation has been implicated in METH neurotoxicity and in the toxicity to environmental stress. In fact, several parallels exist between the effects of METH and environmental stress including Glu and DA release, oxidative damage, and inflammation as indicated by microglial activation and increased cytokines. The matrix metalloproteinases (MMPs) are known to mediate inflammation and have been associated with neurodegenerative diseases such as Alzheimer□s and Multiple Sclerosis. A subclass of MMPs is the gelatinases and includes MMP-2 and MMP-9. These MMPs are known to be expressed in response to

free radicals. Thus, we hypothesized that the serial exposure to chronic stress and METH will increase MMP-2 and MMP-9, and subsequently lead to an increase in neurotoxicity to DA and 5HT terminals. Male Sprague Dawley rats were exposed to chronic unpredictable stress (CUS) or handled for 21 days, treated on day 22 with METH (5 mg/kg x 4; every 2 hr, ip) or saline, and killed on day 23. Results showed an increase in MMP-2 and MMP-9 in the hippocampus following the serial exposure to CUS and METH. Ongoing studies are investigating the effects of CUS and METH on MMPs in other brain regions as well as the involvement of MMPs in neurotoxicity. Supported by DA07606 and DA16866

W-9 **NEONATAL INTRAHIPPOCAMPAL INJECTION OF THE HIV-1 PROTEINS GP120 AND TAT: DIFFERENTIAL EFFECTS ON NEUROBEHAVIOR AND THE RELATIONSHIP TO STEREOLOGICAL HIPPOCAMPAL MEASURES**. S Fitting[1], RM Booze[2], U Hasselrot[1], CF Mactutus[1]; [1]Prog. in Behav. Neuroscience, Dept. of Psychology , University of South Carolina, Columbia, SC 29208-0000, [2]Dept. of Physiology, Pharmacology, & Neuroscience, University of South Carolina, School of Medicine, Columbia, SC 29208-0000.

The present study sought to determine the potential role of HIV-1 proteins on neurobehavioral development and the relationship with the assessed long-term effects of the Tat and/or gp120 on the rat hippocampus. Male pups of 13 Sprague-Dawley litters were bilaterally injected on postnatal day (P)1. Every litter contributed an animal to each of four treatment condition: VEH (0.5µl sterile buffer), gp120 (100ng), Tat (25µg) or combined gp120+Tat (100ng+25µg). Tat revealed an overall effect on many of the behavioral assessments early in development as well as on preattentive processes and spatial memory in adulthood. Gp120 had more selective effects on negative geotaxis (P8-10) and on locomotor activity (P94-96). Potential interactive effects of gp120 and Tat were noted on the early reflex development of negative geotaxis. At ~7 ½ month morphology assessment was conducted by using design-based stereology to quantify the total cell number in five hippocampal subregions [granual layer (GL), hilus of the dentate gyrus (DGH), cornu ammonis fields (CA)2/3, CA1, and subiculum (SUB)] (Fitting et al., 2007). A relationship between early reflex development and estimated cell number in the adult hippocampus was indicated by simple regression analyses. In addition, 81% of the variance of the distribution of searching behavior in the probe test was explained be estimated number of neurons and astrocytes in the DGH. Collectively, these data suggest that the DGH may participate in the spatial memory alterations observed in adulthood consequent to neonatal exposure to HIV-1 proteins. Supported by DA013137, DA014401, HD043680

W-10 **DOPAMINE MODULATES HIV INFECTION IN MACROPHAGES**. PJ Gaskill[1], TM Calderon[1], EA Eugenin[1], JW Berman[1]; [1]Department of Pathology, Albert Einstein College of Medicine, Bronx, NY 10461-0000.

HIV infection of the CNS can result in a variety of neuropathological complications that affect a significant number of HIV positive individuals. One of the major pathological hallmarks of these complications is trafficking and accumulation of monocyte derived macrophages (MDM) in the CNS. HIV induced neurological complications are also compounded by dopaminergic drugs, such as methamphetamine and cocaine, that synergistically increase the pathology of HIV infection in the CNS. Studies have shown that increased extracellular dopamine increases viral expression and neuropathology in SIV infected macaques, but the mechanisms for this enhancement remain unclear. As MDM are the principal targets for HIV in the CNS, it is important to examine the role of these cells in dopamine mediated enhancement of neuropathogenesis. We hypothesize that dopamine is able to modulate the functions of both uninfected and infected macrophages in the CNS, altering the susceptibility of macrophages to HIV infection and enhancing neuropathogenesis. Our data support this hypothesis, demonstrating that dopamine treatment of MDM during HIV infection increases viral replication. This could be mediated through interaction with the endogenous dopamine receptors DR 1, 2, 3, and 4, that we show to be expressed on MDM. We also demonstrate MDM endogenously express the dopamine transporter. Additionally, we show that dopamine increases the activation of signaling molecules in MDM, in particular ERK 1/2, and that the modulation of ERK 1/2 signaling is mediated through a D2-like receptor dependent mechanism. Supported by NIMH, NINDS

W-11 **ENDOGENOUS MORPHINE IN SH-SY5Y CELLS AND IN MOUSE CEREBELLUM**. E Glattard[1], A Muller[1], O Taleb[1], V Kemmel[1], G Roussel[1], MH Metz-boutigue[1], D Aunis[1], Y Goumon[1]; [1]Physiopathology of the Nervous System, INSERM Unit 575, STRASBOURG, 67084.

Endogenous morphine has been characterized in numerous cells and tissues and its structure shown to be identical to morphine from poppy. Its biosynthesis pathway, that derived from dopamine, was recently elucidated in a human neuroblastoma cell line, suggesting its implication in brain physiology. Recently, using the adrenal chromaffin cell model, we have reported the presence of morphine-6-glucuronide (M6G), an analgesic morphine-derived molecule, in secretory granules and in their secretion products, suggesting that endogenous alkaloids might represent neuroendocrine factors. The implication of endogenous alkaloids in the central nervous system was investigated using microscopy, molecular biology and electrophysiology. We report that (i) M6G and morphine, as well as UDP-glucuronosyltransferase 2B (UGT2B) that produce M6G, are present in the human neuroblastoma SH-SY5Y cells, (ii) that morphine is secreted upon nicotine stimulation via a Ca2+-dependent mechanism and

(iii) that morphine and M6G at concentration as low as 10-10M evoke specific naloxone-reversible membrane currents. In order to extend these observations at the brain level, microscopy and proteomic approaches were applied to detect the presence and estimate the quantity of endogenous morphine in mouse brain. Morphine was detected in hippocampus, cortex, olfactory bulb and cerebellum. In the cerebellum, morphine-immunoreactivity is present in GABA-basket cells and their nerve termini that innervate Purkinje cell bodies. Together, our studies suggest that endogenous morphine may represent a new brain modula

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W-12 **STUDY OF THE VARIATION OF MORPHINE LEVELS DURING SEPSIS IN HUMAN**. E Glattard[1], A Muller[1], I Welters[2], T Lavaux[3], D Zhang[1], D Aunis[1], MH Metz-boutigue[1], F Schneider[3], Y Goumon[1]; [1]Physiopathology of the Nervous System, INSERM U575, STRASBOURG, 67084, [2]School of Clinical Science, University of Liverpool, Liverpool, L69 3GA, [3]Medical resuscitation - Hautepierre Hospital, University Louis Pasteur, STRASBOURG, 67098.

Endogenous morphine has been found to be expressed in numerous mammalian cells and tissues. Recently, morphine biosynthesis has been shown in human polymorphonuclear (PMN) and mononuclear cells. Several studies have linked endogenous morphine presence to stress situations. Endogenous morphine increase was found to occur in the blood after cardiovascular bypass and other type of invasive surgery. In addition, lipopolysaccharid (LPS) administrations to rats have been shown to increase the amount of circulating and brain endogenous morphine. We have investigated the presence of endogenous morphine in sepsis using the model of the human PMNs and a clinical studie. Using confocal laser microscopy, molecular biology and proteomic tools, we report that (i) endogenous morphine is located in lactoferrine-containing vesicles of PMNs from healthy donors and (ii) that morphine is secreted upon LPS or IL-8-stimulations. In order to determine the clinical relevance of these observations, the presence of endogenous morphine was studied in vivo on patients with systemic infections. Our clinical study shows that endogenous morphine plasma concentrations statistically change during sepsis in comparison with controls. Our data indicate for the first time that endogenous morphine is present in the blood during sepsis and may derive, in part, from PMNs.

W-13 **GLIAL PROGENITORS ARE TARGETS OF HIV-1 TAT AND INTERACTIVE OPIOID EFFECTS**. YK Hahn[1], S Zou[1], AJ Bruce-keller[2], KF Hauser[1], PE Knapp[1]; [1]Department of Anatomy & Neurobiology, Virginia Commonwealth University, Richmond, VA 23298-0000, [2]Division of Basic Research, Pennington Biomedical Research Center, Baton Rouge, LA 70808-0000.

It is clear that the function/survival of mature neurons and glial cells can be affected

by HIV protein exposure, and that co-exposure to opioids can exacerbate many responses. However, knowledge about the direct or indirect effects of HIV and viral proteins on CNS progenitors is more limited. HIV effects on precursor survival or function might, over time, alter the balance of glia and neurons in the adult CNS, and could be of critical importance for pediatric HIV patients whose CNS is still developing. An isolated progenitor cell population derived from E16 mouse cortex and treated with HIV-1 Tat and/or morphine showed a transient upregulation in caspase-3 activity that peaked between 8-12 hrs of exposure and disappeared by 30 hrs. This population contains multiple types or stages of progenitors, many of which express mu-opioid receptors and therefore may be targets of combined HIV/opioid signaling. Using immunohistochemistry and well-defined cell- and stage-specific markers (e.g. nestin, A2B5, olig-2, CD44, Nkx2.2) we determined that active caspase-3 was upregulated in several types of progenitors, suggesting that HIV/opioid signaling may be toxic or lethal to immature CNS cells at specific stages of development. Additionally, caspase-3 activity was increased by Tat and enhanced by morphine in differentiating, but immature, O4+ oligodendrocytes derived from these progenitors. Morphine effects on caspase-3 and TUNEL staining are presently being evaluated in progenitors within adult transgenic mice expressing Tat1-86 regulated by an inducible GFAP promoter. Supported by DA19398, DA024461

W-14 **MORPHINE INCREASES HIV EXPRESSION IN THE BRAIN OF MICE INFECTED WITH CHIMERIC HIV: POSSIBLE ROLE IN HIV NEUROPATHOGENESIS**. J Kelschenbach[1], A Borjabad[1], G Bentsman[1], P Lu[3], H Gelbard[3], S Chang[2], DJ Volsky[1]; [1]Molecular Virology Division, Columbia University, New York, NY 10019-0000, [2]Department of Biology, Seton Hall University, South Orange, NJ 07079-0000, [3]Department of Neurology, University of Rochester, Rochester, NY 14627-0000.

Drugs of abuse are a major risk factor in HIV transmission, and in vitro studies indicate that drugs of abuse may also contribute to HIV neuropathogenesis, but direct proof in vivo is lacking. Work with SIV indicates that morphine can facilitate SIV infection and brain disease; however such studies have not been possible with HIV because its tropism is limited to humans. We have overcome this limitation by constructing EcoHIV, a chimeric HIV, which recognizes native receptors on mouse cells and can infect mice. One inoculation of mice with EcoHIV establishes widespread infection that lasts for months and includes viral entry into the brain and changes reminiscent of early HIV brain disease in humans. Here we utilize the EcoHIV model to study the effects of morphine on HIV replication in the CNS. B6129F2J mice were exposed to morphine for 72 hours, followed by stereotaxic injections of EcoHIV into the brain to establish acute CNS infection. HIV but not UV-inactivated virus established efficient infection throughout the brain with high levels of viral DNA, RNA, and detectable p24 protein as tested 3-5

days post infection. Morphine tolerant mice showed a 5-fold increase in viral RNA and higher p24 levels in the brain compared to placebo. Notably, virus burdens in the spleen were reduced in morphine tolerant mice. Studies are under way to identify virus target cells and gene expression profiles in the brain of infected, morphine-tolerant mice. We show directly for the first time that morphine may contribute to neuropathogenesis by increasing HIV expression in the brain. Supported by NIDA17618, NIDA22169

W-15 **KAPPA OPIOID RECEPTOR AFFECTS THYMOCYTE DEVELOPMENT THROUGH STAGE-SPECIFIC REGULATION OF IL-7 RECEPTOR EXPRESSION**. M Khimich[1], JM Bidlack[1]; [1]Department of Pharmacology and Physiology, University of Rochester, School of Medicine, Rochester, NY 14642-8711.

M. Khimich and J.M. Bidlack; Dept. of Pharmacology and Physiology, Univ. Rochester, School of Medicine and Dentistry, Rochester, NY 14642. The IL-7 receptor (IL-7R) plays a major role in T-cell development. U50,488, a kappa opioid receptor (KOR) agonist, decreased IL-7R expression level on primary thymocytes. The effect of U50,488 on the expression of IL-7R alpha chain during different stages of thymocyte differentiation was studied. Mice were injected, or extracted T-cells were treated, with U50,488. In both cases, as measured by flow cytometry and RT-PCR, U50,488 decreased the IL-7R levels during stages I and II of thymocyte development, while it did not cause any change in stages III and IV. U50,488 decreased both the number of cells expressing IL-7R and the expression density. The observed changes in IL-7R levels were blocked by the kappa - selective antagonist, nor-BNI. Promoter activity analysis using primary thymocytes and EL4.IL-2 cells revealed that transcription factor AP-1 is responsible for KOR-induced changes in IL-7R expression. These findings suggest that a KOR agonist directly affects thymocyte differentiation in a stage-dependent manner. Supported by NIDA, K05-DA00360, K05-DA04355

W-16 **MORPHINE INHIBITS IMMUNE-CELL INFILTRATION INTO TUMOR MICROENVIRONMENT**. LK Koodie[1], SR Dr. sabita roy[1]; [1]Department of Pharmacology, University of Minnesota, Minneapolis, MN 55455-0000.

Tumor cells within the tumor microenvironment, secretes both proangiogenic factors and chemokines such as MCP-1 for the recruitment of macrophages/monocytes. Infiltrating immune cells are more potent inducers of angiogenesis, generally constitutes a major component of the tumor stroma and possess either tumor growth promoting or inhibiting capabilities. In these studies, placebo and morphine pretreated C56BL/6 mice were injected subcutaneously with LLC tumor cells admixed in matrigel. After 7 days, plugs from placebo and morphine treated mice were removed. Plugs were sectioned and analyzed for angiogenesis as well as macrophage/monocyte infiltration. Our results

suggest that morphine pretreatment inhibited angiogenesis.

W-17 **MORPHINE SYNERGIZES WITH TAT AND S. PNEUMONIAE INFECTION TO INDUCE NEURONAL APOPTOSIS**. A Krishnan[1], J Wang[1], R Charboneau[2], R Barke[2], S Roy[1]; [1]Depatment of Surgery, University of Minnesota, Minneapolis, MN 55455-0000, [2]Department of Surgery, Veterans Affairs Medical Centre, Minneapolis, MN 55417-0000.

Opioid drug abusers have a higher susceptibility to opportunistic infections including S. pneumonie and are a major comorbid factor in NeuroAids. Pneumolysin, a pneumococcal exotoxins has been shown to induce neuronal cell death through a non-classical pathway. Our results show that mice treated with chronic morphine + tat and wild type strains of S. pneumonia showed significantly higher dissemination of bacteria in the CNS when compared to mice treated with morphine + tat and mutant strains of S. pneumonia, although blood levels of bacteria in both groups were similar. Furthermore, only brain sections from the morphine + tat + wild type S. pneumonia treatment mice showed significant apoptosis of neuronal cells. Apoptosis was mediated through a caspase independent apoptotic mechanism by the release of AIF in the cytosol. Our study show for the first time that chronic morphine increases S. pneumococcal dissemination into the CNS and that morphine and pneumolysin acts synergistically with TAT to increase neuronal apoptosis. This could be potential mechanism for the observed prevalence of neuro AIDS in the HIV infected opioid abusers.

W-18 **METHAMPHETAMINE-INDUCED BEHAVIORAL SENSITIZATION AND HYPERTHERMIA ARE ENHANCED IN THE HIV-1 TRANSGENIC RAT**. X Liu[1], M Kass[2], J Peng[1], M Vigorito[2], L Chang[3], SL Chang[1]; [1]Inst. of NeuroImmune Pharmacology, Seton Hall University, South Orange, NJ 07079-0000, [2]Dept. of Psychology, Seton Hall University, South Orange, NJ 07079-0000, [3]Dept. of Medicine, Univ. of Hawaii JABSM, Honolulu, HI 96813-0000.

Using a modified Morris water maze, we showed that the noninfectious HIV-1 transgenic rat (HIV-1Tg), which carries an HIV-1 provirus with a deletion of gag and pol, may be a valid model for studying behaviors related to the neurological dysfunction seen in HIV-1 patients. Methamphetamine (METH) is an addictive psychostimulant which can cause DAergic neurotoxicity, stereotypic behaviors, and hyperthermia. Risk for HIV infection attributable to METH use continues to increase; however the combined effects of HIV and METH are still unclear. To better understand the neurotoxicity associated with HIV and METH use, we compared the behaviors and temperature of 12-13 wk old male HIV-1Tg rats with F344 control animals following daily i.p. injections with 2.5 mg/kg METH for 6 d. Following each injection, motor activity (rearing and grooming) and stereotypic behavior (repeated head movement) were scored for 2 min

at 45, 90, 135, and 180 min and rectal temperature was taken at 0.5, 1, 1.5, 2, 2.5, and 3 h (±2 min). METH increased body temperature and rearing (but decreased grooming) and induced a progressive increase in stereotyped head movement over days (behavioral sensitization). Moreover, the effects on body temperature and sensitization of head movement were enhanced in HIV-1Tg rats. These findings indicate that METH-induced behavioral sensitization and hyperthermia are exacerbated in HIV-1Tg rats. Furthermore, our data suggest that enhancement of METH-induced behavioral sensitization and hyperthermia may be due to injury to DAergic synapses associated with HIV infection. Supported by Supported in part by DA007058, DA016149, and DA019836 to SLC

W-19 **MORPHINE TREATMENT ALTERS BLOOD-TO-BRAIN TRANSPORT OF TH1 CYTOKINES**. JL Lynch[2], WA Banks[1]; [1]GRECC, Verterans Affairs Medical Center, St Louis, MO 63106-0000, [2]Division of Geriatrics- Dept of Internal Medicine, Saint Louis University, Saint Louis, MO 63106-0000.

Interleukin-1 alpha (IL-1a), interleukin-2 (IL-2) and tumor necrosis factor alpha (TNF-a) are Th1 cytokines with potent neuromodulatory effects that are implicated in the etiology and pathogenesis of various psychological and neurological disorders. The finding that chronic morphine treatment alters the Th1/Th2 environment, suppressing Th1 cytokine production, raises the possibility that blood-brain barrier (BBB) transport of cytokines may also be altered by morphine treatment. Here we found that acute morphine treatment (12 mg/kg i.p.) increased blood-to-brain transport of IL-1a while there was no change in blood-to-brain transport of IL-2 and TNF-a. Chronic morphine treatment (48 hr after implantation of 75 mg morphine pellets) and withdrawal from morphine (1 mg/kg of naloxone 48 hr after implantation of 75 mg morphine pellets) did not alter blood-to-brain transport of IL-1a and TNF-a but did increase blood-to-brain transport of IL-2. Typically the permeability of the BBB to IL-2 is dominated by brain-to-blood efflux, with only limited blood-to-brain transport. We found that chronic morphine and withdrawal from morphine did not alter brain-to-blood efflux, but induced a novel saturable blood-to-brain transport system. While IL-1a, IL-2 and TNF-a are all Th1 cytokines, acute and chronic morphine exposure and withdrawal have variable effects on their blood-to-brain transport.

W-20 **MORPHINE SUPPRESSES THE INTERLEUKIN 23-TH17 AXIS MEDIATED INFLAMMATORY RESPONSES TO RESPIRATORY S. PNEUMONIAE INFECTION**. J Ma[1], J Wang[1], R Charboneau[2], R Barke[2], S Roy[1]; [1]Dept. of Surgery, University of Minnesota, Minneapolis, MN 55455-0000, [2]Dept. of Surgery, VA medical Center, Minneapolis, MN 55117-0000.

Streptococcus pneumoniae is a pathogen that causes serious respiratory disease and meningitis in the immune-compromised drug abuse population. Host defense against infection is critically dependent upon host T cells such as Th 17. The immune response of these cells is predominantly a function of effector T cells stimulated by IL-23, but whether IL-23-Th 17 cells axis is required for defense against S. pneumoniae infection is unknown. We tested the hypothesis that S. pneumoniae stimulates the early release of IL-23, leading to increases in the lung Th17 cell population and subsequent IL-17 production that mediates clearance of infection; where morphine treatment inhibits the IL-23 / IL-17 axis and increases susceptibility to S. pneumoniae lung infection. In our study we found a decreased early release of IL-23 in lung tissue, but not in bronchial alveolar lavage (BAL) derived from morphine treated mice after 2 hours of S. pneumoniae infection. Morphine treatment also inhibited the IL-17 protein and mRNA expression in lung tissue at an early stage (2h) of infection. The morphine induced impairment of early IL-23 production and IL-17 protein and mRNA expression are related to decreased clearance of infection and delayed neutrophil migration to the infected lung tissue. Collectively, these studies indicate that (1) the IL-23 / IL-17 axis participates in host defense against S. pneumoniae, and (2) morphine abuse inhibits the pulmonary IL-23 / IL-17 response to S. pneumoniae infection, which leads to a decreased innate and adaptive immune response to S. pneumoniae lung infection Supported by R01 DA12104 and K02 DA015349, P50 DA11806 (S. Roy) and R03 DA023353, T32 DA07097 (J. Wang)

W-21 **SUPPRESSIVE EFFECTS OF MORPHINE ON PRO-INFLAMMATORY WOUND HEALING EVENTS**. JL Martin[1], S Roy[2]; [1]Department of Pharmacology, University of Minnesota, Minneapolis, MN 55455-0000, [2]Department of Surgery, University of Minnesota, Minneapolis, MN 55455-0000.

Opioids, such as morphine, are considered the gold standard in treating pain. Patients prescribed morphine or its derivatives for the management of chronic pain also present with complications such as increased susceptibility to opportunistic infections and inadequate healing of wounds. Basic science and epidemiological studies have documented that morphine exhibits immunosuppressive effects following chronic administration. However, the mechanisms underlying this phenomenon has not been fully understood. Through the standardization of an in vivo murine model, our laboratory has characterized the suppressive effects of morphine on wound healing events in the presence of an infection. A marked decrease in wound closure and wound integrity was seen following chronic morphine administration in the presence of lipopolysaccharides (LPS), as well as, a delay in neutrophil infiltration/decrease in macrophage infiltration into the wound beds. Interestingly, the potent macrophage chemoattractant monocyte chemotactic protein-1 (MCP-1) levels were significantly decreased following chronic

morphine treatment whereas no modulation was seen in the protein levels of the neutrophil chemoattractant macrophage inducible protein-2 (MIP-2). Based on these findings, we are further elucidating the mechanisms by which chronic morphine effects the recruitment and translocation of neutrophils to the site of injury upstream of a chemoattractant gradient formation. We are also exploring additional mechanisms by which macrophage recruitment may be altered following chronic morphine administration. Supported by NIH grants KO1 DA-015349, RO1s DA-12104 and DA-022935

W-22 **ENDOSOMAL TRAFFIC IN HUMAN RENAL PROXIMAL TUBULAR EPITHELIAL CELLS IS CRITICAL FOR HIV-1 ESCAPE AND PROPAGATION IN T CELLS**. J Mikulak[1], H Schmidtmayerova[2], PC Singhal[1]; [1]Medicine, Long Island Jewish Medical Center , New Hyde Park, NY 11042-0000, [2]Immunology and Inflammation, Feinstein Institute for Medical Research, Manhasset, NY 11030-0000.

Renal biopsy data suggest that tubular cells may serve as reservoir for HIV-1. We recently demonstrated that the C-type lectin DEC-205 acts as receptor for HIV-1 in HK-2 cells. Interaction of HIV-1 with DEC-205 results in the capture, rapid internalization of the virus and establishment of nonproductive infection. Here we confirmed that primary Humam Renal Proximal Epithelial Cells (HRPTECs) are permissive to HIV-1 infection and the entry of virus is not dependent on HIV-1 co-receptor tropism. In order to understand the mode of HIV-1 internalization and transmittance in HRPTECs, we studied vesicular transport of HIV-1.To slow down vesicular transport, human renal tubular epithelial cells (HRPTECs) were pre-treated with vesicular pH modulating agents including chloroquine, ammonium chloride, or bafiomycin A followed by HIV-1 infection. HRPTECs were also pre-treated with nocodazole (an inhibitor of transport from early to late endosomes). In HRPTECs pretreated with bafilomycin A, chloroquine and ammonium chloride increased HIV-1 accumulation. Similarly, pretreatment of HRPTECs with nocodazole promoted the accumulation of HIV-1 particles. T cells could rescue HIV-1 from HRPTECs. The infection of T cells was efficiently only in cell-to-cell contact between infected HRPTECs and T cells. Pretreatment of HRPTECs with BFLA-1, CLQ, NH4CL increased the infectivity of HIV in T cells, indicating the endosomes role in escape of HIV-1 in HRPTECs. The trans-infection of virus from HRPTEC to T cells indicate that a small fraction of HIV-1 particles which remain trapped in the endocytic.

W-23 **CHRONIC MORPHINE MODULATES FC-GAMMA RECEPTOR MEDIATED PHAGOCYTOSIS THROUGH A MECHANISM THAT INVOLVES SUPERACTIVATION OF CAMP LEADING TO INHIBITION OF ACTIN POLYMERIZATION**. J. Ninkovic[1], S. Roy[2]; [1]Department of Pharmacology, University of Minnesota, Minneapolis, MN 55455-

0000, [2]Department of Surgery, University of Minnesota, Minneapolis, MN 55455-0000.

Morphine has been known to inhibit innate immunity. Several studies show that morphine inhibits macrophage phagocytosis however, mechanism of this inhibition remains to be explained. Our results indicate that chronic morphine treatment in vitro and in vivo modulates Fc-gamma receptor mediated phagocytosis in murine macrophages. Using fluorescence microscopy and fluorometry, we show decreased internalization of opsonized FITC tagged E. coli bacterial particles as well as opsonized live GFP tagged E. coli following chronic morphine treatment. Microscopic analysis shows that chronic morphine leads to decrease pseudopodia and phagosomal cup formation indicating morphine□s modulation of actin polymerization via small GTP-ases such as Cdc42, Rac and Rho. Forskolin induced elevation of cAMP levels in the macrophage cell line led to suppression of Fc-gamma receptor mediated phagocytosis in morphine and vehicle treated cells. In addition, H89 induced inhibition of PKA resulted in reversal of morphine induced phagocytosis of opsonized bacteria. Together these data indicate that chronic morphine inhibits phagocytosis via a cAMP and PKA dependant pathway, ultimately affecting actin polymerization. Ongoing studies will further elucidate the mechanism of morphine induced decrease in bacterial internalization and its role in modulation of actin polymerization. Supported by National Institutes of Health Grants RO1 DA12104, RO1 DA022935, KO2 DA015349, P50DA11806 (to S.R.) and T32 DA07097 (to J.N.).

W-24 **DELTA-9-TETRAYDROCANNABINOL-TRIGGERS MAST CELL DEGRANULATION THROUGH ACTIVATION OF CANNABINOID RECEPTORS**. MS Pandey[1], DR Venkatesh hedge[1], DR Mitzi nagarkatti[1], DR Prakash nagarkatti[1]; [1]Dept. of Pathology and Immunology, School Of Medicine,University of South Carolina, Columbia, SC 29209.

Chronic marijuana smoking is known to trigger airway inflammation. It is paradoxical that delta-9-tetraydrocannabinol (THC) an important psychoactive component of marijuana has been shown to mediate systemic immunosuppression, while at the same time triggering inflammation in the lungs. In the current study, we tested the hypothesis that THC may activate mast cells and cause their degranulation thereby triggering neutrophil infiltration and inflammation. We demonstrated that various cannabinoids, including THC augment ?-hexosaminidase release, which is a marker of mast cell activation in rat mast cell line (RBL-2H3), murine mast cell line (MC/9) and isolated primary peritoneal mast cells. Mast cells were shown to express CB1 and CB2 receptors. Moreover, we observed that i.p. or intranasal administration of THC in C57BL/6 (WT) mice resulted in dramatic infiltration of neutrophils. CB1 or CB2 receptor selective agonists also induced neutrophil infiltration. Moreover, administration of CB1 or CB2 select antagonists caused significant blocking, of ?-hexosaminidase release

from mast cells. In conclusion, marijuana THC can activate tissue mast cells in vivo leading to dramatic neutrophil-mediated inflammation Supported by Supported by NIH grants: R01AI053703, R01ES09098, R01 AI058300, R01DA016545, R01HL058641 and P01AT003961).

W-25 **PDGF IS NEUROPROTECTIVE AGAINST GP120 TOXICITY**. F Peng[1], H Yao[1], S Buch[1]; [1]Physiology Department, University of Kansas Medical Center, Kansas City, KS 66160-0000.

HIV-1 envelope glycoprotein gp120 has been implicated in mediating neuronal apoptosis, a hallmark feature of HIV-associated dementia (HAD). Mitigation of the toxic effects of gp120 could thus be a potential mechanism for reducing HIV toxicity in the brain. In this study we hypothesized that PDGF could protect the neurons against gp120-mediated apoptosis. SH-SY5Y cells treated with gp120 exhibited increased cell death with concomitant loss of neurites and increased cell rounding. Pre-treatment with PDGF-BB, however, reduced gp120-associated neurotoxicity and rescued neurite outgrowth. Additionally, gp120-mediated activation of caspase-3 was also significantly reduced in cells pretreated with PDGF-BB. Anti-apoptotic effects of PDGF-BB were also confirmed by monitoring levels of anti & pro-apoptotic genes, Bcl-xL & Bax, respectively. Furthermore, PDGF-mediated protection against gp120 involved the PI-3 kinase/Akt pathway. Taken together these findings lead us to suggest that PDGF-BB could be considered as a therapeutic agent that can mitigate gp120-mediated neurotoxicity in HAD.

W-26 **ALCOHOL ABUSE AND OXIDATIVE STRESS IN MACAQUE MODEL OF AIDS**. A Perez casanova[1], V Rivera amill[1], K Husain[1], R Noel[1], A Kumar[2]; [1]Depart. Microbiology - Biochemistry & Pharmacology, Ponce School of Medicine, Ponce, PR 00716-2347, [2]School of Pharmacy - Division of Pharmacology, University of Missouri-Kansas City, Kansas City, MI 64108-2792.

Oxidative stress is well recognized in HIV infection but the association with alcohol abuse is largely unknown. We studied the interactive effect of alcohol abuse and Simian Immunodeficiency Virus/Simian Human Immunodeficiency Virus (SIV/SHIV) infection on plasma and peripheral blood lymphocytes (PBL) oxidant/antioxidant balance in rhesus macaques. Malondialdehyde (MDA), a marker of lipid peroxidation was significantly elevated (2-fold) after infection on plasma and PBL in the experimental group. Glutathione (GSH) were levels depleted (50%) after infection on both samples in alcohol-dependent monkeys. Nitrate oxide (NO) levels significantly increased (3-fold) after week 5 of infection on PBL in both groups but significantly more (40%) in the alcohol exposed group. Activities of superoxide dismutase (SOD) and catalase (CAT)

were reduced (20%) after infection on plasma in alcohol abuse animals. Interestingly, in the PBL, the enzymatic activities increased (3-fold) after infection in alcohol monkeys. Our results exposed strong support for more severe oxidative stress in alcohol monkeys. There was a notable relationship between more severe disease and rate and extent of MDA increase, which may indicates that a combination of alcohol abuse and viral infection leads to more severe disease through a greater disruption in oxidant/antioxidant homeostasis. Supported by 5 RO1 AA015045-3, MBRS-SCORE 5S06GM008239-21, P20 RR-016470 and G12RR0030500

W-27 **REGULATORY T CELLS MODULATE SYNUCLEIN MICROGLIAL NEUROTOXIC ACTIVITIES**. AD Reynolds[1], T Wang[1], I Kadiu[1], P Ciborowski[1], HE Gendelman[1]; [1]Dept. of Pharmacology and Exp. Neuroscience, University of Nebraska Medical Center, Omaha, NE 68198-0000.

Microglial inflammatory processes play principal roles in nigrostriatal degeneration during Parkinson□s disease (PD). This can occur, in part, by microglial activation following engagement to misfolded and oxidized alpha-synuclein released from dying dopaminergic neurons. Qualitative changes in the microglial proteome and secretory constituents induced upon stimulation with nitrated alpha-synuclein consisted of reactive oxygen species production, altered glutamate metabolism, and increased proinflammatory cytokine, redox-active, enzyme, cytoskeletal, and regulatory proteins linked to neurotoxic mechanisms. Nitrated alpha-synuclein microglial mediated neurotoxicity was associated with oxidative stress and cathepsin B activity. CD4+CD25+ regulatory T cells attenuated nitrated alpha-synuclein induction of microglial inflammatory and neurotoxic responses, whereas effector T cells showed no protective effect. We conclude that substantive microglial immune modulation occurs in PD that can be harnessed for therapeutics.

W-28 **NEUROIMMUNE MECHANISMS OF HEROIN-INDUCED ALTERATIONS IN INOS EXPRESSION**. TB Saurer[1], SJ Ijames[1], DT Lysle[1]; [1]Department of Psychology, University of North Carolina at Chapel Hill, Chapel Hill, NC 27599-0000.

Opioid drugs such as morphine suppress a number of immune parameters indirectly by acting through ?-opioid receptors in the brain. Recent findings from our laboratory indicate that morphine inhibits splenic natural killer (NK) cell activity through a centrally mediated pathway that requires the activation of dopamine D1 receptors in the nucleus accumbens shell. Additionally, evidence suggests that the dopamine-dependent effects of morphine on immune status are mediated peripherally via the sympathetic peptide transmitter neuropeptide Y (NPY). The goal of the present study was to determine whether similar neuroimmune mechanisms mediate the effects of heroin, a more

commonly abused opioid, on the production of inducible nitric oxide synthase (iNOS) in response to lipopolysaccharide (LPS) in vivo. The results show that microinjection of the dopamine D1 antagonist SCH-23390 into the nucleus accumbens shell abrogated heroin□s suppressive effect on iNOS mRNA expression in spleen, liver, and lung tissues. Furthermore, the peripherally acting NPY Y1 receptor antagonist BIBP3226 also prevented heroin-induced decreases in LPS-induced iNOS expression. Collectively, these findings indicate that dopamine receptors located in the nucleus accumbens mediate the effects of heroin on iNOS production in vivo and further suggest that heroin-induced increases in nucleus accumbens dopamine alter immune responses to LPS by facilitating NPY release from sympathetic nerves. Supported by NIDA: DA13371; DA00334; DA019323

W-29 **CHARACTERIZATION OF THE PERIPHERAL CANNABINOID RECEPTOR 2 (CB2) TRANSCRIPTIONAL START SITE AND TRANSCRIPT USAGE IN B-CELLS**.
TA Sherwood[1], L Nong[1], M Agudelo[1], C Newton[1], R Widen[1], TW Klein[1]; [1]Molecular Medicine, University of South Florida, Tampa, FL 33612-0000.

Marijuana cannabinoids, the endocannabinoids, and cell receptors have been shown to play an important role in immune regulation particularly as potent modulators of anti-inflammatory cytokines. The predominant cannabinoid receptor believed to be involved in this immune regulation is cannabinoid receptor 2 (CB2), which is expressed most abundantly in B lymphocytes. However, little is known about the regulation of expression of this receptor during immune activation. Utilizing a combination of bioinformatics, 5□ RACE analysis, realtime PCR, and DNA sequencing, we show that mouse splenic B cells express three CB2 transcripts utilizing two different first exons, whereas human peripheral blood B cells express one CB2 transcript utilizing one first exon. Alignment of our sequenced RACE products to either the mouse or human genome, along with the GenBank submitted mRNA sequences, revealed that the transcripts we isolated contained previously unidentified transcriptional start sites (TSS). Our results also show that B cells from mouse and human, at basal activity, preferentially express one transcript, exon 1a (2) in mouse and exon 1 in human. Furthermore, the data illustrate for the first time multiple putative CB2 TSSs utilized in mouse splenic B cells and one putative TSS utilized in human peripheral blood B cells. The defining of the receptor gene TSSs in these cells provides clues to the various gene regulatory factors involved in the expression of CB2 during the activation of B cells and therefore provides a greater understanding of the role of this receptor in B cell biolog Supported by NIDA DA019824

W-30 **PKCZETA MEDIATES THE CCR5-INDUCED CROSS-DESENSITIZATION OF THE MU-OPOIOD RECEPTOR**. C Song[1], T Rogers[1]; [1]Fels Institute and Center for Substance Abuse Res, Temple University School of Medicine, Philadelphia, PA 19140-0000.

We have shown that ?-opoiod receptor (MOR) and HIV co-receptor CCR5 exhibit biodirectional cross-desensitization. In this study, we demonstrate that PKC? mediates MOR desensitization following CCR5 activation. Suppression of PKC? activity either by treatment with a PKC? specific pseudosubstrate inhibitor, or with PKC? siRNA, decreases MOR desensitization in cells treated with the CCR5 agonist MIP-1?. Following CCR5 activiation, the phosphorylation of PKC? is increased in a dose and time-dependent manner. Using fluorescent resonance energy transfer techniques (FRET), we found that the interaction between PKC?-CFP and MOR-YFP is enhanced upon CCR5 activation, and this is suppressed by treatment with the PKC? ?pseudosubstrate inhibitor. Taken together, our data indicate that PKC? participates in CCR5-induced MOR desensitization, suggesting a potential role in the elevated perception of pain caused by the production of chemokine agonists in inflammatory respones. Supported by NIH grants: DA-14230, DA-16544, P30DA-13429, and DA-06650

W-31 **BOTH INTERLEUKIN-1BETA AND MORPHINE UP-REGULATE MU OPIOID RECEPTOR EXPRESSION IN U87 MG CELLS**. L Staikos[1], J Peng[1], SL Chang[1]; [1]Institute of NeuroImmune Pharamacology, Seton Hall University, South Orange, NJ 07079-0000.

Interleukin-1beta (IL-1beta) is a proinflammatory cytokine that exerts its actions via the toll-like IL-1 receptor (IL-1R1). The presence of IL-1beta in the brain has been shown to be involved in neurodegeneration. Morphine, a potent analgesic agent, exerts its effects through the mu opioid receptor (MOR). We have demonstrated that the expression and actions of IL-1beta in the rat brain are altered in the morphine tolerant state, and others have shown that anti-IL-1beta antibody can abolish the immunomodulatory effects of morphine. Astrocytes play an important role in regulating both the function and survival of neurons. U87 MG cells are astrocytoma cells which express IL-1R1. In this study, we used U87 cells to study the possible signaling crosstalk between IL-1beta and morphine. We compared MOR expression in U87 Mg cells with that in SH-SY5Y neuroblastoma cells and human promyelocytic leukemia HL-60 cells using quantitative real time RT-PCR. At the basal level, MOR expression in U87 cells was lower than that in SH-SY5Y cells, but comparable to that in HL-60 cells. The functionality of the MOR in U87 cells was demonstrated by morphine□s inhibition of forskolin-induced intracellular cAMP as determined by radioimmunoassay. Morphine treatment up-regulated the MOR in U87 cells. Additionally, treatment with IL-1beta also

up-regulated MOR mRNA levels in a dose dependent manner in U87 cells. We are currently examining the possible clinical implications of crosstalk between the MOR- and IL-1R1-mediated pathways. Supported by This work was partially supported by DA007058, DA016149, and DA019836 to SLC

W-32 **THE CONDITIONED EFFECTS OF HEROIN ON NITRIC OXIDE ARE MEDIATED BY DOPAMINE D1, NOT D2, RECEPTORS WITHIN THE BASOLATERAL AMYGDALA**. JL Szczytkowski[1], DT Lysle[1]; [1]Department of Psychology, University of North Carolina at Chapel Hill, Chapel Hill, NC 27599-0000.

Heroin induces alterations in a number of immunological parameters including alterations in nitric oxide production. We have shown that heroin induced suppression of inducible nitric oxide synthase (iNOS) can be conditioned to environmental stimuli associated with drug administration. iNOS is the enzyme responsible for the production of nitric oxide which is known to be involved in host defense. Recent studies in our laboratory have shown that the basolateral amygdala (BLA) plays a critical role in the conditioned effects of heroin on iNOS and the present study investigates the role of dopamine signaling with the BLA on these effects. Dopamine is known to be involved in the rewarding effects of opiates and other drugs of abuse and has also been shown to play a role in the conditioned effects of these drugs. Rats were given five conditioning trials in which they received an injection of heroin (1 mg/kg) immediately upon placement into a conditioning chamber. Rats were then re-exposed to the conditioning chamber ten days later without further drug administration. Prior to re-exposure, rats received intra-BLA microinfusions of either the dopamine D1 antagonist, SCH23390 or the D2 antagonist, raclopride. Analyses using real-time RT PCR indicate that blockade of D1, but not D2, receptors in the BLA reverse the effect of heroin associated environmental stimuli on iNOS expression. This study is important because it is the first to demonstrate that the conditioned effects of heroin on iNOS are mediated through dopamine D1 receptors within the BLA. Supported by NIDA DA13371

W-33 **HIV-INFECTED MACROPHAGES MEDIATE NEURONAL APOPTOSIS THROUGH MITOCHONDRIAL GLUTAMINASE**. CH Tian[1], N Erdmann[1], H Peng[1], JL Zheng[1]; [1]Department of Pharmacology and Neuroscience, University of Nebraska Medical Center, Omaha, NE 68198-5880.

A significant number of patients infected with human HIV-1 suffer cognitive impairment ranging from mild to severe HIV-associated demetia (HAD). Mononuclear phagocyte dysfunction is thought to play an important role in the pathogenesis of HAD. Glutamate neurotoxicity is triggered primarily by massive Ca2+ influx arising from over-stimulation of the NMDA subtype of glutamate receptors. The underlying mechanisms,

however, remain elusive. We have tested the hypothesis that mitochondrial glutaminase in HIV-infected macrophages is involved in converting glutamine to glutamate. Our results demonstrate that the concentration of glutamate in HIV-1 infected conditioned media (HIV-MCM) is dependent on glutamine dose, and HIV-MCM mediates glutamine-dependent neurotoxicity. These results indicate HIV-infection mediates neurotoxicity through glutamate production. In addition, glutamate-mediated neurotoxicity correlated with caspase activation and cell cycle re-entry. Inhibition of mitochondrial glutaminase diminished the HIV-induced glutamate production, and attenuated NMDA over-stimulation and subsequent neuronal apoptosis. These data implicate mitochondrial glutaminase in the induction of glutamate-mediated neuronal apoptosis during HIV-associated dementia, and provides a possible therapeutic strategy for HAD treatment.

W-34 **KAPPA OPIOID AGONISTS INHIBIT NF KAPPA B ACTIVATION IN THE U-937 HUMAN MONOCYTE CELL LINE**. CM Tipton[1], JM Bidlack[1]; [1]Pharmacology and Physiology, University of Rochester School of Medicine, Rochester, NY 14642-0000.

The kappa opioid receptor (KOR) has been linked to the regulation of immune function in a variety of cell types. In the current study, the U-937 human monocyte cell line was used to analyze the relationship between KOR activation and Nuclear Factor kappa B (NFkB) activation. Luciferase-reporter assays were used in U-937 cells to demonstrate that KOR activation by each of the agonists, U50,488, U69,593, and enadoline inhibited LPS and TNF-alpha-induced NFkB activation. This inhibition was blocked by the kappa-selective antagonist, nor-BNI. In addition, U-937 cells were analyzed for the expression of the TNF-alpha mRNA following LPS stimulation with and without kappa agonist preincubation. U-937 cells were activated with LPS for 15 to 90 minutes, and TNF-alpha mRNA expression was measured using quantitative real time RT-PCR. Increases in TNF-alpha mRNA were inhibited by 1-hr preincubation with kappa agonists in a concentration-dependent manner. Supported by NIDA T32 DA07232 and K05 DA00360

W-35 **NEUROPATHOGENIC EFFECTS OF HIV-1 TAT AND MORPHINE ON ASTROCYTE CELLS**. A Toro[1], E Rivera[1], R Noel[1]; [1]Biochemistry department, Ponce School of Medicine, Ponce, PR 00731-0000.

Disease progression and neurodegeneration associated with Human Immunodeficiency Virus (HIV-1) infection can be worsened by opioid drug abuse. Tat protein through cytokine upregulation may play a direct role in neuropathogenesis which is related to monocyte infiltration into the CNS. We want to demonstrate the relationship between morphine and HIV-1 Tat truncated and full length forms in cytokine production alteration and cell death. Astrocyte cell lines were exposed to morphine and Tat to

measure cytokine expression changes and apoptosis. Neuroblastoma cell viability was measured after treating the cells with astrocyte conditioned media. Both HIV-1 Tat forms and morphine increase astrocyte mRNA levels of proinflammatory cytokines IL-6 and IL-8. In contrast, both Tat forms and morphine diminish IL-6 protein levels but increase astrocyte IL-8 secretion. The truncated form of Tat produces more astrocyte cell apoptosis than full length form as found in T cells by others. In our cell model, Tat and morphine are combined cause a minor increase in cell death, in contrast to what has been published by others. Treatment of neuroblastoma cells with astrocyte conditioned medium increases cell apoptosis levels. Our results indicate that the pro-inflammatory cytokine secretion induced by Tat and morphine may be involved in the cell apoptosis promoting neurodegeneration. Supported by P20 RR-016470, NIH/NIGMS GM008239 and G12RR003050

W-36 **HIV VPR AND OPIOIDS INTERACTION IN RAT ASTROCYTES ALTER CHEMOKINES GENE REGULATION**. L Torres[1], E Rivera[1], L Maldonado[1], RJ Noel jr.[1]; [1]Biochemistry Department, Ponce School of Medicine, Ponce, PR 00716-0000.

Chemokines are known to have an indispensable role on HIV neuropathology. They induce cell migration and control interaction between different cells types. RANTES, TNF and MCP-1 are chemokines that are released from glial cells in HIV infection in response to viral proteins. Astrocytes are the major glial cell type in the CNS responsible for the production of such cytokines. It is already known that cytokines production can be altered by some viral proteins and opioids. To determine the combined effect of HIV viral protein R (Vpr) and drug abuse in expression of inflammatory cytokines and receptors, we performed Vpr transfection of rat astrocytes and added morphine. Total RNA was extracted and analyzed using a PCR array and real time RT-PCR. We found that Vpr upregulated the expression of MCP-1, RANTES, TNF alpha and TNFR2; when combined with morphine, MCP-1 was synergistically increased, while RANTES showed no effect and TNF alpha and TNFR2 were modestly reduced. The data support the importance of Vpr as a neurotoxin with combined pathology in HIV-1 infected drug abusers. Supported by NCRR 003050, NIGMS 008239

W-37 **NATURAL KILLER CELLS SUPPRESS FULL CYCLE HCV INFECTION OF HUMAN HEPATOCYTES**. SH Wang[2], L Ye[1], X Wang[1], L Song[1], YJ Wang[1], XY Huang[2], CX Huang[2], WZ Ho[1]; [1]Division of Allergy and Immunology, The Children's Hospital of Philadelphia, Philadelphia, PA 19104-0000, [2]Renming Hospital of Wuhan University, Wuhan University, Wuhan, 430060.

The role of natural killer (NK) cells in controlling hepatitis C virus (HCV) infection has

not been fully delineated. We examined NK cell-mediated non-cytolytic effect on HCV infection of human hepatocytes. Supernatants (SN) from NK cells cultures (both primary NK cell and NK cell line, NK92) inhibited HCV JFH1 infection of human hepatocytes, which was evidenced by decreased expression of both HCV RNA and protein. This anti-HCV activity of NK SN could be largely abolished by antibody to interferon(IFN)-gamma. The contribution of IFN-gamma to NK cell-mediated anti-HCV activity is also supported by the observation that NK SN enhanced the expression of signal transducer and activator of transcription-1 and 2 (STAT1, STAT2), the nuclear factors that are essential for the activation of IFN-mediated antiviral pathways. In addition, NK SN-treated hepatocyte expressed higher levels of IFN-alpha/beta than the control cells, providing a mechanism for IFN-gamma-mediated anti-HCV effect. This enhancement of intracellular IFN-alpha/beta expression by IFN-gamma was the results of the induction of IFN regulatory factors 3 and 7. These data provide direct evidence at cellular and molecular levels that NK cells have a key role in suppressing HCV infection of and replication in human hepatocytes. Supported by NIH DA 12815

W-38 **NEONATAL EXPOSURE TO TAT INCREASES EXPRESSION OF INFLAMMATORY CYTOKINE IL-1BETA**. KM Webb[1], S Fitting[2], MY Aksenov[2], RM Booze[2], CF Mactutus[2]; [1]Dept. of Pharmcology, Physiology and Neuroscience, University of South Carolina School of Medicine, Columbia, SC 29208-0000, [2]Behavioral Neuroscience Program, Dept. Psychology, University of South Carolina, Columbia, SC 29208-0000.

Globally there are 33 million HIV infected people of whom 2.5 million are children. Although NeuroAIDS is more commonly associated with HIV-positive adults, children are increasingly affected by this debilitating disease. In addition to neuronal damage induced by toxic proteins of HIV, research is increasingly indicating that the inflammatory response initiated by the immune system in an infected brain can further damage neurons and disrupt function. Previous studies from our lab of an animal model of pediatric NeuroAIDS indicate that they are delayed in meeting developmental milestones, as are human children infected with HIV. In this project we evaluated the early immune response to the presence of HIV toxic proteins Tat and gp120 in this same model. We injected saline, 25ug Tat or 100ng gp120 in a 1 ul volume into the unilateral hippocampus. All injections were performed in male, postnatal day (PD) 1 Sprague-Dawley pups. On PD3 animals were sacrificed and the hippocampus was removed by microdissection for RNA isolation. Purified RNA was transcribed into cDNA templates, then probed for the expression of inflammatory cytokines Il-1beta, TNF-alpha, Il-6, Ikappa-beta and NFkappa-beta, as well as the housekeeping gene GAPDH by Multiplex Polymerase Chain Reaction (MPCR). MPCR results indicate a significant increase in the expression of Il-1? in Tat-treated animals. These findings correlate well

with clinical observations of the immune response in the brain that show an increase in Il-1beta expression due to the activation of macrophages, the carrier of HIV into neural tissue.

W-39 **PDGF PROTECTS MIDBRAIN NEURONS AGAINST TAT-INDUCED NEUROTOXICITY**. HH Yao[1], FW Peng[1], N Dhillon[1], S Buch[1]; [1]Department of Physiology, University of Kansas Medical Center, Kansas City, KS 66160-0000.

HIV-associated dementia (HAD) is often characterized by neuronal loss induced by human immunodeficiency virus type 1 (HIV-1) transactivator protein Tat. Therefore, inhibition of neuronal loss has been regarded as a prospective approach for treating HAD. In the present study, we explored the protective effect of the neurotrophin platelet-derived growth factor (PDGF) against Tat-induced neurotoxicity. Rat primary midbrain neurons pretreated with PDGF followed by Tat exposure demonstrated enhancement of neuronal protection. PDGF treatment also led to an increase in dendrite-length as demonstrated by tyrosine hydroxylase (TH) staining. Mechanism of neuroprotection by PDGF involved de-activation of caspase-3 and inhibition of p38/ MAPK phosphorylation induced by Tat. This was also corroborated by the pharmacological inhibitor of PI3K inhibitor LY294002, which abrogated the protective effect of PDGF in Tat-exposed neurons. Furthermore, we also demonstrated that PDGF-mediated protection also involved activation the downstream survival target, Akt. Taken together, these findings reveal for the first time that PDGF protects neurons against Tat-induced toxicity via down-regulation of caspase-3 activity and up-regulation of the Akt signal transduction pathways. Targeting these signaling molecules may have potential as novel therapeutic strategies for mitigating neuronal damage associated with HAD. Supported by DA020392-01,MH6296901, MH068212, MH072355

W-40 **ALCOHOL ENHANCES COMPLETE HEPATITIS C VIRUS INFECTION OF HEPATOCYTES**. Y Zhou[1], SH Wang[2], L Ye[2], X Wang[2], YJ Wang[2], DJ Zhou[1], WZ Ho[2]; [1]Virology Dept., Wuhan Centers for Disease Control & Prevention, Wuhan, 430022, [2]Division of Allergy &Immunology, Joseph Stokes, Jr, University of Pennsylvania School of Medicine, Philadelphia, PA 19104-0000.

Alcohol and HCV infection most likely act synergistically to promote the development and progression of liver disease. However, there is little direct evidence at cellular and molecular levels about the impact of alcohol on complete HCV infection of and replication in human hepatocytes. The availability of the robust HCV infectious cell model has provided a unique opportunity to investigate the direct interactions between alcohol, innate immunity and HCV infection. Alcohol, when added to naive human hepatocytes, significantly inhibited intracellular interferon (IFN)-? expression. This

alcohol-mediated suppression of intracellular IFN-? ?expression was associated with an increase in HCV replication in human hepatocytes. The cells pretreated with alcohol showed increased susceptibility to HCV infection. Moreover, alcohol compromised the anti-HCV activity of recombinant IFN-?. Investigation of the mechanism responsible for the alcohol action revealed that alcohol inhibited expression of signal transducer and activator of transcription 1 (STAT1), the nuclear factor essential in the activation of IFN-gamma in human hepatocytes. These findings provide direct in vitro evidence that by inhibiting intracellular IFN-?-mediated innate immunity alcohol may have a cofactor role in facilitating HCV infection and persistence in the human hepatocytes.

POSTER SESSION 2

- General Session -

T-1 **RETROGRADE AND ANTEROGRADE TRANSPORT OF HIV PROTEIN GP120 IN THE CENTRAL NERVOUS SYSTEM**. F Ahmed[1], L Macarthur[1], I Mocchetti[1]; [1]Neuroscience, Georgetown University Medical Center, Washington, DC 20057-0000.

Neurodegeneration and gliosis are prominent features of human immunodeficiency virus (HIV) dementia complex (HAD). In these individuals, neurodegeneration occurs in uninfected neurons at anatomical sites that are often distant from the site of viral replication. Therefore, to design new therapies, it is crucial to uncover the molecular and cellular mechanisms underlying HIV neurotoxicity. The HIV protein gp120 is neurotoxic and mimics some of the pathological alterations seen in HAD. Retrograde transport of this protein appears to be crucial for its neurotoxic effect in the central nervous system (CNS). However, it is still unclear whether gp120 can be transported anterogradely and how transport occurs in the peripheral nervous system. To determine whether gp120 is transported retrogradely and anterogradely, we injected gp120IIIB together with Fluoro-Ruby into the rat superior colliculus. GP120 was retrogradely transported with Fluoro-Ruby along a direct pathway from the superior colliculus to the retina. In addition, gp120 immunoreactivity was found in the lateral geniculate nucleus and in the occipital cortex (anterograde transport). In contrast, gp120 injected into the sciatic nerve was not transported to dorsal root ganglia, suggesting that gp120 axonal transport occurs only in the CNS. CNS neurons transporting gp120 exhibited activated caspase-3 within 3 days.

Transported gp120 was often associated with reactive gliosis at a later time. These results indicate that axonal transport of gp120 might play a role in HIV-1-mediated widespread neuronal cell death in the CNS. Supported by NIH/NS040670

T-2 **DIFFERENTIAL SURVIVAL OF HIV-1 TAT EXPOSED RAT FETAL NEURONS**. MY Aksenov[1], MV Aksenova[1], CF Mactutus[1], RM Booze[1]; [1]Department of Psychology, University of South Carolina, Columbia, SC 29208-0000.

HIV-1 Tat causes neurodegeneration in primary cultures of fetal human and rodent neurons. In this study we report that Tat toxicity in primary cultures of rat fetal neurons is limited to a certain part of neuronal population. Binding/uptake of Tat 1-86 by some neurons in rat fetal midbrain cell cultures exposed to 50 nM Tat 1-86 was increasing during the first hour of treatment and was followed by activation of caspase 9 and the increase of MCP1 (CCL2) immunoreactivity. Total caspase activity was increased in rat fetal midbrain cell cultures treated with Tat for 2 and 24 hours. Increased MCP1 (CCL2) expression was evident in Tat-treated cell cultures until the 48-hour time point. Cell viability declined within the period of time between 24 and 72 hours of Tat treatment and was accompanied by gradual decline of the cell bound Tat immunoreactivity. Despite the presence of neurotoxic Tat 1-86 in the medium, 71% of neuronal population was able to survive the continuous exposure to Tat. The survivors were found to be resistant to repeated Tat treatments. Many neurons in the residual part of Tat-exposed cell cultures expressed NMDA receptor complexes, which are believed to play a key role in Tat neurotoxicity. The expression of NR1 and NR2B NMDAR subunits was not different from non-treated controls, but levels of NR2A immunoreactivity were significantly decreased. Our results suggest that different subunit composition of NMDA receptor complexes may, at least in part, influences the sensitivity of neurons to HIV-1 Tat toxicity. Supported by NIH grants DA 11337, DA 09160, DA 84401, HD 043680

T-3 **FREE RADICAL-DRIVEN ACTIVATION OF MACROPHAGE BY OPIATES AND HIV TAT**. AJ Bruce-keller[1], J Turchan-cholewo[4], JN Keller[1], PE Knapp[3], KF Hauser[3]; [1]Division of Basic Research, Pennington Biomedical Research Center/LSU, Baton Rouge, LA 70808-0000, [2]Department of Anatomy and Neurobiology, Virginia Commonwealth University, Richmond, VA 23298-0000, [3]Department of Pharmacology and Toxicology, Virginia Commonwealth University, Richmond, VA 23298-0000, [4]Department of Anatomy and Neurobiology, University of Kentucky, Lexington, KY 40536-0000.

HIV-1 patients who abuse opiate-based drugs have increased rates of HIV dementia, which may be related to brain inflammation. To better understand how opiates could derange HIV-related brain inflammation, the effects of morphine and HIV-Tat on free radical production and redox-based inflammatory signaling was measured in

macrophages and microglia. Data show that administration of Tat stimulated the production of free radicals, which are key to the subsequent production and release of cytokines. However, co-administration of morphine with Tat significantly increased Tat-induced free radical production and intracellular oxidation, while significantly decreasing cytokine release. Finally, data show that the ability of free radicals to control inflammatory signaling in microglia/macrophages may be based on oxidative alterations to the multicatalytic proteasome and/or on differential recruitment of free radical□producing enzymes to lipid raft signaling platforms. Together, these findings suggest that free radical production in monocytic cells could be a key mechanism controling cytokine release, and that this mechanism is controlled by opiates. Supported by RO1 NS046267, P20 RR15592, and P01 DA19398

T-4 **BRAIN GLUTAMATE AND GLUTATHIONE AS OXIDATIVE STRESS MARKERS IN HIV AND METH ABUSE**. L Chang[1], J Panee[1], A Yanagihara[2], S Buchthal[1], M Ricardo-dukelow[1], H Nakama[1], M Berry[1], T Ernst[1]; [1]Neuroscience and Magnetic Resonance Research, John A. Burns SOM, Univerisity of Hawaii at Manoa, Honolulu, HI 96813-0000, [2]Pacific Biosciences Research Center, University of Hawaii at Manoa, Honolulu, HI 96813-0000.

Oxidative stress may be involved in neuronal injury in HIV patients with or without history of methamphetamine (METH) abuse. Serum glutathione (GSH), an antioxidant, were shown to be decreased in HIV, as well as in postmortem brains of METH users. Whether oxidative stress (measured by GSH) will show additive effects in HIV subjects who abuse METH is unknown. Using a novel proton magnetic resonance spectroscopy (1H MRS) technique (MEGA-PRESS), we determined brain GSH concentrations in HIV patients with or without METH abuse, and in controls. We additionally measured the precursor glutamate (GLU) levels using another new MRS technique (TE-averaged PRESS). Lastly, we assessed whether the extracellular (CSF) levels were related to brain levels of GLU and GSH, and whether these changes were related to other MRS markers of brain injury and cognitive performance. Exploratory studies included a multidimensional battery of assays (non-enzymatic antioxidant capacity, the activities of several antioxidant enzymes involved in the detoxification of superoxide and peroxides, and breakdown products from DNA and lipids) to evaluate the antioxidant status of these subjects Findings may provide insights regarding the mechanism(s) of brain injury that include decreased reuptake of GLU by HIV infected dysfunctional glia (as shown by elevated myoinositol), and hence decreased recycling of GLU via glutamine back to glutamatergic neurons, hence decreased synthesis of GSH, oxidative stress, neuronal injury and cognitive deficits, despite antiretroviral treatment in these HIV subjects. Supported by NIH (K24-DA16170; K02-DA16991; 1U54-NS056883)

T-5 **ENDOSOMAL INHIBITOR CHLOROQUINE USURPS THE HIV-REPLICATION IN NEUROGLIAL CELLS**. A Chauhan[1], V Theophilus sunder[1]; [1]Dept. Pathology, Microbiology and Immunology, University of South Carolina School of Medicine, Columbia, SC 29209-0000.

The HIV-infection in brain is primarily in macrophages and microglia, which leads to neurological complications. Despite the presence of chemokine but absence of CD4 receptors on astrocytes and neurons, infection is reported to be minimally productive in astrocytes, but neurons are barely infected. The enveloped viruses including some retroviruses enter into the target cells via pH-dependent endocytosis. Notably, the HIV enters through pH-independent pathway using CD4-receptor and either CCR5 or CXCR4 as coreceptor followed by fusion with the host membrane and enter into cytoplasm. However, HIV entry in non-CD4 is not yet clearly understood. We investigated HIV-infection in neuro-glial cells using endosomal inhibitors. Following HIV infection, minimal productive infection was seen in astrocytes but not in neurons. Further, we found the infection in astrocytes independent of CD4, CXCR4, CD11a, and mannose receptors. The infection started on day 5 and peaked between 10 -15 day post infection. Interestingly, in long term follow-up astrocytes did not show trans-infection to vicinity cells, however, upon co-culture with lymphocytes transmitted the infection robustly. Further, we investigated the HIV infection in neuroglial cells in the presence of anti-malarial drug chloroquine, an endosomal inhibitor used in malaria endemic areas. Intriguingly, chloroquine treatment increased the infection 16-20 fold in astrocytes and neurons irrespective of viral strain either R5 or X4, suggesting an endocytosis mediated entry of HIV in non-CD4 cells. In parallel, we used another endosomal inh Supported by NIH

T-6 **ROLE OF SUMOYLATION IN COMPLEMENT COMPONENT C3 GENE REGULATION**. P Datta[1], J Rappaport[1]; [1]Center for Neurovirology, Dept. of Neuroscience, School of Medicine, Temple University, Philadelphia, PA 19122-0000.

Uncontrolled synthesis and activation of the complement component C3 by the proinflammatory cytokine IL-1 beta in the brain can lead to neuroinflammation and neurodegeneration. We have demonstrated earlier that IL-1 beta induces C3 synthesis in astrocytes and monocytes in a C/EBP dependent manner. We now investigated whether IL-1 beta and C/EBP mediated C3 promoter activation is regulated by the enzymes of the SUMO pathway. Our studies demonstrate that overexpression of E3 SUMO ligase, PIASy in astrocytes and monocytes inhibits not only IL-1 beta but also C/EBP-delta and C/EBP-beta isoform LAP mediated C3 promoter activation. On the contrary, overexpression of SUMO protease, SENP1 further enhances the transcriptional response of the C3 promoter by IL-1 beta and C/EBP. These observations demonstrate that modification of the transcription factor C/EBP by sumoylation and desumoylation play significant role in C3 gene regulation. Supported

by NIH

T-7 **INTERPLAY OF COCAINE & HIV-1 IN BLOOD BRAIN BARRIER DISRUPTION: ROLE OF PLATELET-DERIVED GROWTH FACTOR**. N Dhillon[1], X Zhu[1], S Callen[1], K Kim[1], S Buch[1]; [1]Dept. of Physiology, University of Kansas Medical Center, Kansas City, KS 66103-0000.

Epidemiological and clinical studies on HIV-infected persons suggest that drug abuse including cocaine, may enhance HIV-replication and incidence of HIV-encephalitis (HIV-E) in the early stage of AIDS. 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. Although cocaine effects on the enhancement of viral neuroinvasion through the BBB have been studied earlier, the interplay of HIV and cocaine in the disruption of BBB and the mechanisms involved in this process remain unclear. In our previous studies, we have demonstrated increased expression of PDGF in the brains of macaques with SHIV-encephalitis (SHIV-E). Furthermore, neuropathological changes in the brains were found to correlate with increased PDGF expression by perivascular macrophages. Our recent studies have shown that that exposure of BMVECs and infected monocytes to cocaine also leads to an increased expression of PDGF-B chain and its cognate receptors in these cells. Further we demonstrate that PDGF can disrupt the integrity of the BBB in an in vitro model system. These findings coupled with the known function of PDGF as an inducer of MCP-1 (a key chemokine critical for the recruitment of monocytes in the CNS), suggests that the combinatorial effects of cocaine and HIV can enhance the BBB permeability by modulating the expression of PDGF. Supported by DA020392-01, MH62969-01

T-8 **MECHANISMS BY WHICH OPIATES INDUCE SYNERGISTIC INCREASES IN CYTOKINE RELEASE TRIGGERED BY HIV-1 TAT EXPOSED ASTROCYTES**. NE El-hage[1], AJ Bruce-keller[2], PE Knapp[1], KF Hauser[1]; [1]Department of Pharmacology and Toxicology , Virginia Commonwealth University, Richmond, VA 23298-0000, [2]Division of Basic Research, Pennington Biomedical Research Center/LSU, Baton Rouge, LA 70808-0000.

We assessed sites where opioids and HIV Tat intracellular signals converge to synergistically increase MCP-1, RANTES and IL-6 release by astrocytes. Initial studies screening a large number of transcription factors indicated that NF-?B/Rel was critical for Tat or opiate plus Tat induced increases in cytokine production by astrocytes. Subsequent studies showed that opiates increases NF-?B activity through PI3K/Akt and [Ca2+]I. The studies showed that the inhibition of PI3K blocks Tat ± morphine-induced activation of p-AKT, increases in [Ca2+]i, and the production of MCP-1, IL-6 and TNF-?.

Similarly, sequestering [Ca2+]i by using the cell permeate Ca2+ chelator, BAPTA/AM, also blocked Tat protein ± morphine-evoked MCP-1, IL-6 and TNF-? release, while artificially elevating [Ca2+]i by increasing extracellular [Ca2+] reversed this effect. Importantly, inhibiting PI3K or chelating Ca2+ abrogated Tat ± morphine stimulated NF-?B activation and nuclear translocation. Conversely, the effects of morphine could be mimicked by artificially increasing [Ca2+]i, while opiates no longer affected NF-?B activation at peak [Ca2+]I . Together, our results suggest astrocytes are significant in orchestrating opiate and HIV-1 induced inflammation in the CNS. In particular, signaling through the phosphatidylinositol 3-kinase (PI3K)/Akt pathway and intracellular Ca2+ ([Ca2+]i), trigger increases in NF-?B activation and nuclear translocation that are responsible for the synergist. Supported by NIDA-DA19398

T-9 **CCL5 MEDIATES MORPHINE AND TAT-INDUCED INCREASES IN CCL2 AND GLIAL REACTIVITY**. N El-hage[1], AJ Bruce-keller[2], PE Knapp[1], KF Hauser[1]; [1]Department of Pharmacology & Toxicology, Virginia Commonwealth Univ. School of Medicine, Richmond, VA 23298-0000, [2]Division of Basic Research, Pennington Biomedical Research Center/LSU, Baton Rouge, LA 70808-0000.

CCL5/RANTES is hypothesized to be important for regulating CCL2/MCP-1 expression by astrocytes, which in turn regulates Tat ± morphine-induced reactive gliosis in the CNS. To delineate the intercellular events involved and the role of CCL5 in driving HIV-1 Tat ± opiate-induced glial reactivity and CCL2 production associated with HIV-1 encephalitis (HIVE), wild type and CCL5(-/-) mice were injected intrastrially with vehicle or 25 mg HIV-1 Tat1-72 and treated with placebo or morphine (s.c. implants) for 7 d. Proportional changes in astroglia (GFAP), macrophages/microglia (F4/80), CCL2, and CCL5 immunoreactivity were assessed approximately 200-500 microns from the injection site. Synergistic increases in astrocytes and macrophages/microglia were seen in CCL5(+/+) mice with combined morphine exposure and intrastriatal Tat that were absent in CCL5(-/-) mice. Moreover, Tat or combined morphine and Tat exposure markedly increased the proportion of astroglia and macrophages/microglia possessing CCL2 immunoreactivity compared to vehicle-injected controls, while increases in CCL2 colocalization in astrocytes and macrophages/microglia were attenuated in CCL5 null mice. The results indicate that CCL5 is an important mediator of the reactive gliosis and inflammatory caused by Tat ± morphine, and suggests that CCL5 acts in part by regulating inflammatory increases in CCL2 in HIVE. Supported by NIH NIDA DA 19398

T-10 **ENDOCANNABINOIDS AND THE PATHOGENESIS OF MURINE HIV-1 ENCEPHALITIS**. S. Gorantla[1], J. Hutter[1], J. Poklis[2], E. Makarov[1], A. Holguin[1], H. Dou[1], C. Murrin[1], B. Martin[2], H. Gendelman[1], L. Poluektova[1]; [1]University of Nebraska Medical Center, Dept. of Pharmacology and Exp. Neuroscience, Omaha, NE 68198-5880,

[2]Virginia Commonwealth University , Dept. of Pharmacology and Toxicology, Richmond, VI 23298-0613.

Chronic HIV-1 infection affects behavioral, cognitive, and motor function in its human host. This is governed through immune activated and virus-infected mononuclear phagocytes (MP; blood borne macrophages and microglia) that secrete neurotoxins, and ingress of lymphocytes into the brain. Infiltrating immune cells can facilitate the control of viral infection as well as eliciting blood-brain barrier and the neuronal network disturbances. It was shown that the endocannabinoid (eCB) system can affect immunity and neural function. We analyzed eCBs ligands (2-AG and anadamide) and their receptors (CB1R, CB2R and GPR55) in a mouse model of HIV-encephalitis (HIVE), in which HIV-infected human MP were injected into the basal ganglia of immunodeficient mice reconstituted with human lymphocytes (hu-PBL-HIVE). 2-AG and CB2R expression were increased in HIVE brain tissue. JTE-907, a CB2R selective antagonist/inverse agonist and Gp1a, a selective CB2R agonist significantly reduced brain ingress of human lymphocytes in hu-PBL-HIVE mice and lowered the levels of HIV-1gag expression in affected brain tissues. JTE-907 abrogated cell ingress into brain in hu-PBL animals without encephalitis. These findings were associated with increased expression of CB2R on peripheral human lymphocytes and mouse macrophages. Moreover, both drugs reduced human cell (CD4 and CD8) numbers that expressed CCR5 and PD-1 receptors. Our results suggest that CB2 receptors and their ligands are potential therapeutic candidates for human disease. Supported by NIH/NINDS R21 NS060642, NIH/COBRE P20RR15635

T-11 **OPIATES SYNERGISTICALLY INCREASE GLIOSIS, NEURONAL INJURY, AND SYNAPTIC LOSSES IN THE STRIATUM OF HIV TAT TRANSGENIC MICE**. KF Hauser[1], N El-hage[1], A Nath[2], A Bruce-keller[3], PE Knapp[1]; [1]Department of Pharmacology and Toxicology, Virginia Commonwealth Univ. School of Medicine, Richmond, VA 23298-0000, [2]Department of Neurology, Johns Hopkins Univ., Baltimore, MD 21287-0000, [3]Division of Basic Research, Pennington Biomedical Research Center/LSU, Baton Rouge, LA 70808-0000.

To assess how opiate abuse exacerbates HIV encephalitis (HIVE), wild type and transgenic (tg) mice expressing HIV-1 Tat1-86 regulated by a doxycycline (DOX) inducible, GFAP promoter were continuously treated with placebo, morphine, and/or naltrexone (s.c. implants) ± DOX for 2, 7, or 10 d. Morphine and/or Tat induction caused marked increases in reactive astroglia and macrophages/microglia within 2 days that appeared to be elevated thereafter, as well as sustained elevations in MCP-1 (CCL2) and RANTES (CCL5) mRNA and/or protein levels. Parameters of neuronal injury significantly increased by morphine exposure and Tat-induction included (i) elevations in cleaved caspase 3, and reductions in (ii) dendritic length and (iii) spine density in Golgi-impregnated medium spiny neurons. When dendrite length and spine density are

considered collectively, morphine causes synergistic reductions the number of dendritic spines. Despite significant increases in active caspase 3, dendritic pruning and synaptic culling, there was little evidence of increased neuron cell death as assessed by TUNEL. Thus, within this timeframe, neurons appear to sustain injuries that may lead to functional loss without cell death. Our results indicate that Tat intrinsically causes glial inflammation, neuronal injury, and synaptic reductions associated with HIVE, and further show that opiates exacerbate glial reactivity and neuronal injury through direct interactions with Tat. Supported by NIDA DA19398 and DA18633

T-12 **WIN55,212-2 INHIBITS MICROGLIAL CELL MIGRATION TOWARDS CHEMOKINES AND DOPAMINE**. S Hu[1], W Sheng[1], RB Rock[1], PK Peterson[1]; [1]CIDMTR, Dept. of Medicine, University of Minnesota Medical School, Minneapolis, MN 55455-0000.

The dopaminergic nigrostriatal area is highly susceptible to HIV-1-related brain disease, and experimental evidence suggests that activated microglia recruited to this brain region play a neuropathogenic role. These brain macrophages express not only chemokine receptors but also receptors for neurotransmitters, such as dopamine (DA) and cannabinoids. The chemokines CCL2 and CX3CL1 have been shown to be involved in HIV-1 neuropathogenesis, while little is known about the effects of DA on microglia. The cannabinoids have been reported to have neuroprotective properties through receptor- and non-receptor-mediated mechanisms. In this study we found that human microglia express not only CX3CR1, the specific receptor for CX3CL1, by FACS but also DA receptors, DR1, DR2, DR3 and DR5 by real-time PCR. Using chemotaxis chambers to measure cell migration, we demonstrated that microglia are capable of migrating toward CCL2, CX3CL1 and DA in a concentration-dependent manner and that the addition of DA to CCL2 potentiated microglial cell migration. When pretreated with the synthetic CB1/CB2 receptor agonist WIN55,212-2, migration of microglia toward CCL2 or DA alone or in combination was inhibited in a concentration-dependent manner, while the inactive enantiomer WIN55,212-3 showed no effect. These findings support the concept of cannabinoids as anti-inflammatory agents and suggest that by targeting microglia cannabinoids could have therapeutic potential for HIV-1-related brain disease. Supported by NIH

T-13 **COCAINE MODULATES T CELL ACTIVATION BY HUMAN DENDRITIC CELLS (DC) AND ENHANCES SUSCEPTIBILITY TO HIV**. SM Kiertscher[1], J Zhuo[1], A Harui[1], GC Baldwin[1], MD Roth[1]; [1]Department of Medicine, David Geffen School of Medicine at UCLA, Los Angeles, CA 90095-1690.

Infection by HIV and progression to AIDS involves a complex interaction between the host immune system, HIV, and a number of cofactors. Our work focuses on how

cocaine modulates the immune response to HIV and acts as a cofactor in the pathogenesis of HIV/AIDS. Previously, we demonstrated that activation by DC leads to the upregulation of CD4 and chemokine coreceptors on responder T cells and enhances their susceptibility to HIV infection. We hypothesized that cocaine may modulate this process, resulting in increased infection by HIV. Exposure to cocaine during the differentiation of human monocytes into DC altered their cell surface phenotype and cytokine/chemokine profile to favor increased coreceptor expression and Th2-related gene expression. To determine if this modulation of DC phenotype affects their ability to stimulate T cell responses and therefore impacts HIV infection, we stimulated allogeneic T cells with either cocaine-exposed or control DC and an X4 HIV reporter virus in an MLR assay. Activation by both control and cocaine-exposed DC induced HIV infection in the responding T cells, however the frequency and magnitude of HIV infection was enhanced in the T cells stimulated by cocaine-exposed DC. Similar results were obtained in an adenoviral antigen presentation assay, in which antigen-exposed DC were used to stimulate autologous T cells. These results suggest that cocaine influences the immune response to HIV by impacting the phenotype and function of human DC, the characteristics of DC-activated T cells, and their subsequent susceptibility to HIV infection. Supported by NIDA/NIH #DA023386

T-14 **METHAMPHETAMINE ENHANCES HUMAN IMMUNODEFICIENCY VIRUS INFECTION OF MACROPHAGE**. H Liang[3], X Wang[2], H Chen[3], L Song[2], L Ye[2], SH Wang[2], YJ Wang[2], WZ Ho[2]; [1]Center for AIDS Research , Guangxi Medical University, Nan Ning, Guang Xi, 530021, [2]Division of Allergy & Immunology, The Children□s Hospital of Philadelphia, Philadelphia, PA 19104-0000, [3]First Affiliated Hospital, Guangxi Medical University, Nan Ning, Guang Xi, 530021.

Methamphetamine (METH), a sympathomimetic stimulant, has been implicated in human immunodeficiency virus (HIV) disease. However, there is a lack of direct evidence that METH promotes HIV infection of the target cells. This study examined whether methamphetamine has the ability to enhance HIV infection of macrophages, the primary site for the virus. METH treatment resulted in a significant and dose-dependent increase of HIV reverse transcriptase activity in macrophages. This METH-mediated increase of HIV infectivity in macrophages could be blocked by the dopamine D1 receptor antagonists (SCH23390 and SKF83566). Investigation of the underlying mechanisms for the METH action showed that METH had the ability to upregulate the expression of HIV entry coreceptor CCR5 on macrophages. Additionally, METH inhibited the expression of endogenous interferon alpha and signal transducer and activator of transcription1 in macrophage. These findings provide a direct in vitro evidence to support the possibility that METH may have a co-factor role in the immunopathogenesis of HIV infection. Supported by NIH (NIDA12815 and NIDA22177)

T-15 **UNIQUE ROLE OF ARMS IN NEUROTROPHIN-MEDIATED ACTIVATION OF NF-KB AND NEURONAL PROTECTION AGAINST HIV-1 ENCODED GP120.**. SB Maggirwar[1], LF Sniderhan[1]; [1]Department of Microbiology and Immunology, University of Rochester Medical Center, Rochester, NY 14642-0000.

Activation of the transcription factor NF-?B is a key feature of the signaling triggered by neurotrophins, and this activity has been shown to be critical for neuronal survival examined in in vitro models of neuroAIDS. However, the precise mechanism by which neurotrophins activate NF-?B is not well understood. In an effort to dissect this process, we analyzed the role of brain-derived neurotrophic factor (BDNF) and its receptor, TrkB, on NF-?B activity in cortical neuronal cultures. Here we show that TrkB expression is developmentally regulated in primary cortical cultures, and expression levels correlate with the ability of BDNF to activate NF-kB. This action of BDNF requires functional Trk since addition of K252a, a potent inhibitor of Trk, completely abrogated NF-kB activation. We further report that the activation of NF-?B by BDNF requires normal expression of ARMS and its association with Trk. Notably, an overexpression of ARMS augments BDNF-induced NF-?B signaling in cortical neurons, while dissociation of TrkB:ARMS interaction by overexpression of the membrane -spanning region of ARMS blocks NF-?B signaling. These analyses were further extended through the generation of an ARMS siRNA expressing lentivirus and essentially supported our conclusions. Finally, we found that ARMS is required for BDNF to protect neurons from toxicity induced by HIV-1 encoded gp120. Thus, we propose that the interaction of TrkB with ARMS is essential for NF-kB activation by neurotrophins and subsequent neuroprotection against candidate HIV-1 neurotoxins. Supported by RO1 NS054578, PO1 MH64570

T-16 **HUMAN IMMUNODEFICIENCY VIRUS-1 PROTEIN TAT REDUCES THE K+-EVOKED RELEASE OF STRIATAL DOPAMINE: IMPLICATIONS FOR VMAT-2 FUNCTION**. WF Maragos[1], RL Self[1], S Theodore[1], AG Deaciuc[2], LP Dwoskin[2], WA Cass[3]; [1]Neurology, University of Kentucky, Lexington, KY 40536-0000, [2]College of Pharmacy, University of Kentucky, Lexington, KY 40536-0000, [3]Anatomy and Neurobiology, University of Kentucky, Lexington, KY 40536-0000.

In the brain, basal ganglia structures are highly susceptible to infection with the human immunodeficiency virus-1 (HIV) as well as injury by methamphetamine (MA), a drug often abused by HIV-1 infected individuals. We have previously shown that the HIV protein, Tat, interacts synergistically with MA to damage the basal ganglia dopaminergic system. Synergy only occurred when MA was administered 24 hrs after injections of Tat, suggesting that Tat had an unknown □priming effect□. In this study, we measured K+-evoked dopamine (DA) release in rats 24 hrs following intrastriatal injections of Tat. Compared to the contralateral vehicle-treated striatum, injections of Tat resulted in a 40% decrease in the peak K+-evoked DA release, but this was only observed when

animals were pretreated with nomifensine to prevent DA reuptake by the dopamine transporter. In contrast, Tat had no effect on MA-evoked DA release. As K+-evoked DA release is an index of the vesicular stores of DA, we next examined the effects of Tat on the vesicular monoamine transporter (VMAT-2) function. Compared to vesicles prepared from the vehicle-treated striatum, those prepared from the Tat-treated striatum showed a 35% reduction in [3H]DA uptake. Ceramide, the production of which is stimulated by Tat, inhibited VMAT-2 function in a concentration-dependent manner and inhibition of the ceramide-producing enzyme sphingomyelinase significantly attenuated Tat+MA toxicity. The results of these studies suggest that Tat enhances the toxic potential of MA via a mechanism involving ceramide inhibition of VMAT-2. Supported by DA13144 (WFM), T32-DA16176 (RLS), DA13519 (LPD) and AG17963 (WAC)

T-17 **CSF CYSTATIN C IS DECREASED IN HIV-SEROPOSITIVE WOMEN WITH HAND AND CHRONIC SMOKING**. LM Melendez[1], V Wojna[1], M Plaud-valentin[2], R Skolasky[3], J Lasalde[4]; [1]Specialized Neuroscience Program in NeuroAIDS, University of Puerto Rico Medical Sciences , San Juan, 00935, [2]School of Medicine, University of Puerto Rico Medical Sciences , San Juan, 00935, [3]Orthopedic Surgery, John Hopkins University, Baltimore, MD 21287-7613, [4]Biology, University of Puerto Rico Rio Piedras, San Juan, 00921-0000.

Nicotine is a drug of abuse that has been associated with improvement of cognitive function. Cystatin C is believed to play a protective role against endogenous and pathogen-derived proteases. Cystatin C has been found decreased in the CSF of patients with HIV Neurocognitive Dysfunction (HAND). We hypothesized that nicotine affect the expression of cystatin C in the CNS of patients with HAND. To test this hypothesis, we first investigated the role of cystatin in HAND, and then we evaluated the effect of smoking in cystatin C expression in the CSF of 20 HIV seropositive women: 4 with normal cognition, 9 asymptomatics (A), and 7 with symptomatic cognitive impairment (CI) by ELISA. When stratified by PI as part of antiretroviral therapy, we found that cystatin C concentration decreased significantly from A to CI (Chi-Square 6.618, d.f. 2, p=.037). To evaluate the effect of smoking on cystatin C expression, 26 HIV seropositive women were stratified into three groups: 7 non-smokers, 10 with past-history of smoking, and 9 chronic smokers. We found that there was a significant difference in cystatin C among these three groups (F (2,23 d.f.) = 5.51, p=0.011). Post-hoc testing showed the difference was between non-smokers and chronic smokers (p=0.011). In conclusion, cystatin C is decreased in A and chronic smokers suggesting that both conditions decrease the production of this enzyme. Further studies are needed to evaluate the effect of smoking on cystatin C and cognitive function in this population. Supported by U54NS43011, P20RR1126, G12RR03051

T-18 **EFFECT OF SMOKING IN THE CSF PROTEIN PROFILES FROM HIV-SEROPOSITIVE WOMEN**. LM Melendez[1], V Wojna[1], J Perez laspiur[1], E Rodriguez[1], F Duan[2], J Lasalde[1]; [1]Specialized Neuroscience Program in NeuroAIDS, University of Puerto Rico Medical Sciences Campus, San Juan, 00935, [2]Medical Center, University of Nebraska, Omaha, NE 68198-5800.

Nicotine enhances alpha 7 acetylcholine receptor in mononuclear phagocytes (MP). Smoking correlates with improved cognitive function in the Hispanic women cohort. However, nicotine can also increase HIV replication in vitro. Our hypothesis is that nicotine activation of HIV-infected MP affect CSF protein expression, increasing the production of neuroprotective proteins. To test this hypothesis we initially compared the protein profiles of CSF from 26 HIV seropositive women: 9 chronic smokers, 10 with history of smoking, and 7 nonsmokers characterized for HIV Neurocognitive Dysfunction (HAND) using surface enhancement laser disorption ionization time of flight protein chip mass spectrometry. Using the statistics of Generalized Estimating Equations and false discovery rate, we found significant differences in 12 protein peaks between nonsmokers and chronic smokers (adjusted p value < 0.1). When we compared the CSF protein profiles between nonsmokers and those with history of smoking, we found significant differences in 23 protein beaks between both groups, 21 of which showed significantly increased intensities in the CSF of patients with smoking history (adjusted p value < 0.1). Protein peaks 5876 and 5980 where increased in chronic smokers and remained increased in those with history of smoking. In conclusion, smoking can affect the CSF proteome in HIV infected women with antiretroviral therapy, by increasing two protein peaks in the CSF of patients with history of smoking and in chronic smokers. Supported by U54NS430,P20RR11126,G12RR03051

T-19 **MECHANISMS OF NEURO-AIDS BY HIV-1B AND C CLADES**. MP Nair[1], SK Saxena[2], DJ Feaster[3], JW Rodriguez[4], ZM Saiyed[1], A Diaz-gonzalez[1], I Borodowsky[1], T Samikkannu[1], KB Dakshayani[1], E Provencio-vasquez[5]; [1]Department of Immunology, College of Medicine, Florida International Uni., Miami, FL 33155-0000, [2]Department of Infectious Diseases, Center for Cellular and Molecular Biology, Hyderabad, 500007, [3]Florida International University, Stempel School of Public Health, Miami, FL 33199-0000, [4]Universidad Central del Caribe, School of Medicine, Puerto Rico, 00960, [5]University of Miami, School of Nursing and Health Studies, Miami, FL 33124-0000.

The discovery of multiple subtypes of HIV-1 worldwide has created new challenges to control HIV-1 infection and associated neuropathogenesis. Although previous studies indicate a difference in neuropathogenic manifestations between HIV-1B and C clade infected subjects, the question whether clade B is more neuropathogenic than clade C remains elusive because of the lack of a well controlled, systematic clinical and research studies. Previous studies demonstrate that proinflammatory cytokines and chemokines play a significant role in neuropathogenesis of HIV-associated dementia.

We hypothesize that clade B and C exert differential effects on CNS cells leading to differential gene expression and production of proinflammatroy molecules associated with neuropathogenesis. Primary human monocytes and astrocytes were incubated separately with gp-120 and tat from HIV-1B and C isolates and the RNA was extracted, followed by quantitative real time PCR for IL-6, TNF? and MCP-1 and the supernates were tested by protein array. Results indicate that gp-120 and tat from HIV-1B significantly upregulated proinflammatory molecules, IL-6, TNF? and MCP-1 by primary monocytes and astrocytes compared to gp-120 and tat from HIV-1C in identical culture conditions. This suggests that clade B and clade C proteins differentially modulate the production of neuropathogenic molecules. These studies may help to unravel the differential effect of clade specific infection on neuropathogenesis and may be of therapeutic significance. Supported by NIDA

T-20 **DELTA-9-TETRAHYDROCANNABINOL ATTENUATION OF TH1 RESPONSE IN MICE INFECTED WITH LEGIONELLA PNEUMOPHILA IS CANNABINOID RECEPTOR MEDIATED**. C Newton[1], I Perkins[1], TW Klein[1]; [1]Molecular Medicine, Univ South Florida College of Medicine, Tampa, FL 33612-0000.

Legionella pneumophila causes acute infection in mice mediated by proinflammatory cytokines. The infection induces Th1 immunity characterized by L. pneumophila-specific production of IFN gamma and IgG2a antibodies days after infection. There are also increased blood levels of IL-12 and IFN gamma within hours of infection. Delta-9-Tetrahydrocannabinol (THC) treatment prior to L. pneumophila infection causes a shift from Th1 to Th2. The mechanism of THC treatment involves both cannabinoid receptors- CB1 and CB2. When CB1 (SR141716A; SR1) or CB2 (SR144528; SR2) antagonists were injected prior to THC treatment, the THC-induced suppression of IL-12 and IFN gamma blood levels was attenuated. The same effect was also observed in studies using CB1-/- or CB2-/- mice on C57BL/6 background. IL-12R?2 is required for IFN gamma production. IL-12R?2 message was decreased with THC treatment and this attenuation was CB1-mediated as demonstrated using SR1 with BALB/c mice as well as C57BL/6 and CB1-/- mice. GATA3 transcription factor is important in early T-cell development and is selectively expressed in Th2 cells. GATA3 message levels were elevated with THC treatment and this enhancement involved CB2 as indicated with SR2 in BALB/c mice and C57BL/6 with CB2-/- mice. An examination of NFkB p65 revealed an increase with L. pneumophila and a further enhancement with THC treatment. These results indicated that both receptors are involved with THC induced suppression of Th1 immunity; however, the receptors have different functions in this attenuation. Supported by NIH/ DA03646, AI45169 and DA10683

T-21 **PROLONGED ACTIVATION OF FUNCTIONAL MU-OPIOID RECEPTOR ISOFORM-1 AUGMENTS HIV-1 SUSCEPTIBILITY OF TF-1 BONE MARROW PROGENITOR CELLS BY ENHANCING FORSKOLIN-STIMULATED CAMP ACCUMULATION**. MR Nonnemacher[1], A Banerjee[1], A Alexaki[1], B Wigdahl[1]; [1]Department of Microbiology and Immunology, Drexel University College of Medicine, Philadelphia, PA 19102-0000.

Several studies have suggested that opioids act as a cofactor in enhancing susceptibility to human immunodeficiency type 1 (HIV-1) infection in immune cell populations as well as modulating innate, humoral, and cell-mediated immunity. CD34+ bone marrow progenitor cells are refractile to HIV-1 infection, probably due to their low level expression of HIV-1 co-receptors CXCR4 and CCR5. We have identified functional mu opioid receptor isoform-1 (MOR-1) in the human CD34+/CD38+ TF-1 bone marrow progenitor cell line. Therefore, this cell system was utilized as a model to elucidate the effects of chronic opioid exposure on the alteration of HIV-1 infection. Interestingly, as previously reported in other cellular systems, persistent stimulation of G-alpha(i) opioid receptors on these cells brings about a paradoxical augmentation of adenylate cyclase activity. To this end, experiments reveal that chronic exposure of the CD34+/CD38+ human bone marrow progenitor cell line TF-1 to mu-opioid ligands followed by adenylate cyclase supersensitization upregulates the viral co-receptor CXCR4. This is also accompanied by a concomitant increase in HIV-1 replication, suggesting an increase in HIV-1 susceptibility and/or increased LTR-directed transcription. The specific role of cAMP and the downstream transcription factors involved in these molecular signaling events are currently being investigated. Supported by NIDA/R01 DA19807-03

T-22 **ROLE OF NITRIC OXIDE IN DEFENSE AGAINST TUBERCULOUS MENINGITIS.** MR Olin[2], AG Armien[2], PK Peterson[2], TW Molitor[2]; [1]College of Veterinary Medicine, University of Minnesota, St. Paul, MN 55108-0000, [2]Department of Medicine, University of Minnesota, Minneapolis, MN 55455-0000.

Tuberculous meningitis (TBM) is a serious complication of tuberculosis (TB), which when untreated is almost always fatal. Recently, researchers have described mouse models for the study of TBM and reported lesions and lymphocytic infiltration into the CNS following intracerebral (ic) inoculation of tubercle bacilli. However, these studies failed to detect granulomas, and clinical signs and mortality were not observed. The objective of this project was to establish a murine model of TBM that would mimic the disease in humans. Knowing that murine but not human microglia express iNOS, NOS-/- or wildtype mice were inoculated ic with M. tuberculosis (MTb) H37rv. NOS -/- mice demonstrated neurological signs of TBM and a 62% mortality. In addition, multifocal coalescing epithelioid macrophage aggregations surrounded by lymphocyte infiltration (granulomas) were apparent in the brains of all the iNOS -/-mice. In the brain cortex,

granulomatous inflammation was associated with extension of the lesions into the meninges or ventricular system. Acid Fast bacilli were present within macrophages in affected areas of the brain. No clinical signs, mortality, lesions or bacilli were detected in wildtype mice. This study corroborates the importance of NO in host defense against MTb and suggests that NOS -/- mice are an appropriate model for the study of human TBM. Supported by NIDA/R21DA007097-26A1

T-23 **METHAMPHETAMINE-INDUCED OXIDATIVE STRESS CAUSES DYSFUNCTION OF PRIMARY HUMAN T CELLS**. R Potula[1], B Morsey[1], RM Brodie[1], SH Ramirez[1], Y Persidsky[1]; [1]Dept. of Pharmacology/Experimental Neuroscience, University of Nebraska Medical Center, Omaha, NE 68198-5215.

Illicit drugs such as methamphetamine (METH) alter immune functions and increase susceptibility to infections. Oxidative stress as consequence of METH exposure is thought to contribute to METH-induced neurotoxicity, and may also cause impairment of T cell responses. We therefore explored the idea that METH triggers oxidative stress leading to T cell dysfunction and dysregulation of immune system. T cells are critical for orchestration of immune responses, as activation and proliferation of T cells are characteristic of adaptive immunity. Our results indicated that METH in non-toxic concentrations induced generation of reactive oxygen species (ROS) and nitric oxide (NO) in primary human T cells. METH exposure caused dose dependent decrease of IL-2 mRNA expression, resulted in inhibition of IL-2 production and blocked proliferation by human T cells following TCR stimulation. Exposure of T cells to METH caused significant modulation of genes regulating immune cell activation and T cell surface markers as indicated by the RNA superarray analyses. In summary, METH induced generation of ROS and NO in T cells, decreased cytokine production and T cell proliferation upon TCR cross-linking and differentially regulated genes associated with T cell signaling machinery. These finding suggest that oxidative stress induced by METH could be underlying cause of immune dysfunction leading to accelerated progression of chronic infections (like HIV-1). Supported by Development Grant from the UNMC

T-24 **METHAMPHETAMINE INDUCED BLOOD BRAIN BARRIER (BBB) DYSFUNCTION IS MEDIATED BY DOWN-REGULATION OF TIGHT JUNCTION PROTEINS**. SH Ramirez[1], R Potula[1], A Papugani[1], T Eidem[1], Y Persidsky[1]; [1]Dept. of Pharmacology/Experimental Neuroscience, University of Nebraska Medical Center, Omaha, NE 68198-5215.

Permeability of the BBB was reported in animals exposed to methamphetamine (METH) suggesting that some of METH neurotoxic effects could be the result of barrier disruption. The tight junctions (TJ) connecting endothelial cells is mainly responsible for maintaining barrier function. We explore the possibility that TJ proteins are altered in

primary human brain endothelial cells (BMVEC) in response to METH exposure. Using transendothelial electrical resistance (TEER) to measure barrier function, we demonstrated that METH treatment of BMVEC monolayers resulted in a rapid dose-dependent decrease of TEER (26-45%). A second indicator of barrier compromise is the increase infiltration of immune cells across the endothelium. Drug treatment resulted in two-fold augmentation of monocyte and lymphocyte passage in response to relevant chemokines, CCL2 and CCL5 in a dose-dependent manner. Image analysis of TJ proteins, occludin and claudin-5 staining in a human brain endothelial cell line showed discontinuous junctions and gap formations after METH treatment. Decrease in claudin-5 and occludin levels were also detected by Western blot in the membranous fractions of endothelial cells after METH exposure. Finally, analysis of Rho GTPases (regulating the TJ in BMVEC) appeared to be activated by METH exposure. In summary, we were able to demonstrate for the first time decreased barrier tightness, increased TJ alterations, enhanced monocyte and lymphocyte adhesion/migration after METH exposure to BMVEC and correlate these alterations to the Rho GTPase function in BMVEC and TJ alterations. Supported by a Development Grant from the UNMC

T-25 **GP120 TRIGGERS OXIDATIVE STRESS-RELATED DAMAGE OF HUMAN DOPAMINERGIC NEURONS**. RB Rock[1], S Hu[1], WS Sheng[1], PK Peterson[1]; [1]Center for Infect. Dis. and Micro. Trans. Res., University of Minnesota, Minneapolis, MN 55455-0000.

The dopamine (DA)-rich midbrain is known to be a key target of HIV-1. Studies of simian immunodeficiency virus (SIV)-induced neuropathogenesis recently established that there is a major disruption within the nigrostriatal dopaminergic system characterized by marked depletion of dopaminergic neurons, microglial cell activation, and reactive astrocytes. Using a human midbrain cell culture model, which contains dopaminergic neurons, microglia, and astrocytes, experiments were performed to characterize the damage to dopaminergic neurons induced by gp120 by assessing functional impairment as measured by DA uptake, and to investigate neurotoxicity by assessing apoptosis and oxidative damage. By using this midbrain culture model, we were able to identify the relative sensitivity of dopaminergic neurons to gp120-induced damage, that gp120-induced neuronal damage was manifest as reduced function (decreased DA uptake) and altered morphology (dendrite loss) and viability (apoptosis). We also showed that gp120-induced oxidative damage is involved in this neuropathogenic process. Supported by NIDA/DA020398-01

T-26 **METHADONE ENHANCES HIV-1 INFECTIVITY AND MODULATES THE EXPRESSION OF CXCR4 AND TH1/TH2-TYPE CYTOKINES IN LYMPHOCYTES FROM CHRONIC METHADONE PATIENTS**. JW Rodriguez[1], M. Rodriguez[1], C. Lopez-

cepero[1], R. Hunter[1], M. Nair[2], E. Rios-olivares[1]; [1]Department of Microbiology and Immunology, Universidad Central del Caribe School of Medicine, Bayamon, PR 00960-0000, [2]Department of Immunology, Florida International University, Miami, FL 33199-0000.

Drug addition is a major risk factor for human immunodeficiency virus-1 (HIV-1) infection. Estimates indicate that injection drug users (IDUs) constitute approximately 25% of new HIV-1 cases in the United States and 70% in Puerto Rico. Immunopharmacological and epidemiological studies have indicated that opiates have conditionally variable effects on HIV-1 disease progression. In vitro and in vivo methadone (MTD) experiments have shown suppressive effects on immune function and can enhance HIV-1 infection. However, the immunovirological effect of MTD on lymphocytes from chronic MTD patients is not well characterized. Our laboratory has been evaluating the immunovirological effect of MTD on lymphocytes and its association with HIV-1 infectivity. Lymphocytes from HIV-1 negative MTD patients were acutely infected with HIV-1 and assayed to determine how continuous ex vivo exposure of MTD affects HIV-1 infectivity, the expression of HIV-1 co-receptor (CXCR4), and the production of TH1 (IL-2, IFN-g, TNF-a) and TH2 (IL-4, IL-6, IL-10) cytokines using real-time quantitative PCR and protein array. Our results showed that ex vivo exposure of MTD increases HIV-1 infectivity by 3-folds. In addition, this was also correlated with a significant increase in CXCR4 and TH1/TH2-type cytokine expression. These findings provide a new insight on the immunovirological effects of MTD and raise a major concern on the use of MTD as the drug of choice for the treatment of opiate addiction in HIV-infected patients. Supported by NIH-RCMI G12RR03035

T-27 **DIFFERENTIAL REGULATION OF INDOLEAMINE -2, 3-DIOXYGENASE (IDO) BY HIV-1 B AND C CLADES**. T Samikkannu[1], K Dakshayani[1], MP Nair[1]; [1]Department of Immunology, College of Medicine, Miami, FL 33155-0000.

HIV-1 infection contributes to neuropathogenesis and development of HIV-1 associated dementia. The discovery of multiple subtypes of HIV-1 worldwide has created new challenges to control HIV-1 infection and associated neuropathogenesis. Although previous studies indicate a difference in neuropathogenic manifestations between HIV-1B and C clade infected subjects, the question whether clade B is more neuropathogenic than clade C remains elusive. Previous studies demonstrate that first rat limiting enzyme Indoleamine-2, 3-Dioxygenase (IDO), which converts tryptophan into kynurenine; may play a significant role in neuropathogeneis of HIV- associated dementia. We hypothesize that clade B and C exert differential effects on IDO gene expression and production of kynurenine associated with neuropathogenesis. Human primary astrocytes were cultured separately with clade B and C TAT (5-100 ng/ml) and RNA was extracted, followed by quantitative real time PCR for IDO gene expression. The culture supernatants were assayed for kynurenine while the cell lysates were

analyzed for IDO enzyme. Results indicate that clade B TAT protein significantly upregulated IDO gene expression, IDO enzyme activation and increased kynurenine production compared to clade C TAT. This suggests that clade B and C tat proteins differentially modulate the production of enzyme IDO that may be associated with increased neuropathogenesis reported in HIV-1B infection. These studies may help to unravel the mechanisms of clade specific infection of HIV-1 neuropathogenesis. Supported by NIDA

T-28 **ANTIJED: A POSSIBLE THERAPEUTIC AND IMMUNOMODULATORY FOR JAPANESE ENCEPHALITIS**. SK Saxena[1]; [1]Infectious Diseases Group, Centre for Cellular and Molecular Biology, Hyderabad (AP), 500007.

AntiJEd, a low molecular weight dithiol, has been described as an immunomodulator and modifier of diverse biological actions in human and animal models and has also been shown to be effective in several diseased conditions. Therefore we studied the therapeutic aspect of AntiJEd in providing inhibition of Japanese encephalitis virus (JEV) infection. AntiJEd tested at various doses (10-100 mmol/kg) revealed that administration at low concentration (10 mmol/kg; i.p.) on alternate days prolonged the average survival time (AST) of mice infected with lethal dose of JEV (102 LD50, i.c.) and delayed progression of the disease. The low dose also provided >80% survival in sub-clinical (105 LD50, i.c.) JEV infection. Administration of AntiJEd to JEV-infected mice enhanced the inducible nitric oxide synthase (iNOS) activity in brain and level of serum tumor necrosis factor-a (TNF-a). We have recently demonstrated the production of nitric oxide (NO) via induction of iNOS activity is meditated by circulating macrophage-derived factor (MDF), which may be responsible for the delayed progression of the disease. AntiJEd mediated inhibition of JEV is believed to involve the augmentation of protective role of MDF as evidenced by the observation that pretreatment with anti-MDF antibody significantly decreased the AST of mice and together with the inhibition of iNOS activity. Interestingly, AntiJEd alone did not stimulate iNOS and TNF-a in mock-infected normal mice. These results show that AntiJEd may have possible therapeutic and immunomodulatory role during JEV infection.

T-29 **MODULATION OF HUMAN NEURAL PRECURSOR CELL PROPERTIES BY HIV-1 TAT**. P Seth[1]; [1]Molecular and Cellular Neuroscience, National Brain Research Centre, Manesar, 122050.

Central nervous system (CNS) infection by HIV causes irreversible damage to the neurons via apoptosis that result in neurodegeneration, generally accompanied by progressive motor and cognitive dysfunctions and other dementia like symptoms, collectively designated HIV-associated dementia (HAD). Although role of glial cell mediated neuronal damage, and direct neuronal cell death following HIV infection is well

documented, only few reports define the involvement of neural precursor cells in CNS infections. Adult neurogenesis has been demonstrated in several neurodegenerative diseases, including human immunodeficiency virus (HIV-1)-associated dementia, which warrants the need to investigate if HIV or its viral proteins modulate human neural precursor cell properties and may diminish the chance to rescue or replenish the damaged neuronal pool by the process of neurogenesis. We hence investigated the effect of the HIV-1 transactivating protein (Tat) on cell survival, growth, proliferation, as well as differentiation potential of human neural precursor cells. Using a novel human neurosphere cell culture system developed by us, we observed that HIV-1 viral protein Tat modulates the proliferation of human neural precursor cells as assessed by decrease in the size as well as lower incorporation of Brdu in the developing human neurospheres as well as other parameters studies by us. We believe our study provides new insights into understanding of HIV-1 induced alterations in the cellular and molecular mechanisms of neurogenesis in human CNS progenitor cells. Supported by Department of Biotechnology

T-30 **WIN55,212-2 INHIBITS PRODUCTION OF CX3CL1 BY HUMAN ASTROCYTES: INVOLVEMENT OF P38 MAP KINASE**. W Sheng[1], S Hu[1], RB Rock[1], PK Peterson[1]; [1]CIDMTR, Dept. of Medicine, University of Minnesota , Minneapolis, MN 55455-0000.

CX3CL1 (fractalkine) has been shown to be neuroprotective but also play a role in HIV-1-associated dementia pathogenesis. Production of CX3CL1 in the CNS is mainly from neurons and astrocytes. The neuroprotective property of cannabinoids has been reported to involve CB1/CB2 receptor- or non-receptor-mediated mechanisms, and the expression of CB1/CB2 receptors has been demonstrated in microglia and astrocytes. In this study we have found upregulated CX3CL1 production by human astrocytes stimulated with interleukin (IL)-1b in a concentration-dependent manner. The expression of CX3CL1 mRNA peaked at 6h after IL-1b stimulation. When pretreated with the synthetic CB1/CB2 receptor agonist WIN55,212-2 prior to IL-1b stimulation, CX3CL1 production by astrocytes was downregulated while the inactive enantiomer WIN55,212-3 had a minimal effect. Using CB1/CB2 receptor specific antagonists, SR141716A and SR144528, respectively, we found that inhibition of CX3CL1 production by WIN55,212-2 was blocked by both antagonists suggesting a CB1/CB2 receptor-mediated action. IL-1b treatment significantly induced p38 and ERK1/2 (p44/42) MAP kinase phosphorylation, and WIN55,212-2 inhibited IL-1b-induced p38 MAPK phosphorylation only. Using inhibitors of p38 MAPK (SB203580) or MEK1/MEK2 (U0126), only SB203580 inhibited IL-1b-stimulated CX3CL1 production suggesting a p38 MAPK-dependent mechanism. Our results support the notion that synthetic cannabinoids have anti-inflammatory properties and that they may have therapeutic potential for certain

neuroinflammatory disorders. Supported by NIH

T-31 **MULTIPLEX HIV DNA ASSAY TO ASSESS HIV DNA IN PBMC SUBSETS**. B Shiramizu[1], M. Agsalda[1], D. Troelstrup[1]; [1]HACRP; John A. Burns School of Medicine, University of Hawaii, Honolulu, HI 96816-0000.

Background: Current therapy effectively treats HIV-1-infection but has not eliminated the prevalence of neurocogntive problems. Therapy has little effect on viral DNA compared to viral RNA. Because PBMC HIV DNA levels correlate with disease progression, we hypothesize that HIV DNA may be an important player in neuropathogenesis. We previously demonstrated that high PBMC HIV DNA correlated with HAD. We developed a new multiplex PCR assay for HIV DNA quantification to assess HIV DNA in PBMC subsets. Methods: Two plasmids were designed with copies of the HIV gag and b-globin genes. Equal amounts of both plasmids were mixed to make plasmid dilutions from 10(7) to 10(1) copies of each gene. HIV measurement was also completed on control cell lines and compared to results after whole genome amplification. Multiplex reactions and singleplex reactions were performed in triplicate on all the plasmid dilutions and cell lines. PBMC subsets (CD14+; CD14+/CD16+; CD14+/CD16-; PBMC) were assayed for HIV DNA. Results: The multiplex assays resulted in equivalent amplifications and equivalent threshold amplification cycles (no significant differences between the two assays with p-values between 0.8-0.9 for all tests) as the respective singleplex reactions with 100% concordance; with sensitivity of 10 copies per 10(6) cells. Higher HIV DNA was found in CD14+/CD16+ subsets in subjects with HAD. Conclusions: Multiplex real-time PCR efficiently measures HIV DNA and detects HIV DNA in PBMC subsets. The increased throughput makes the assay a potential tool for large studies. Supported by NIH

T-32 **ACTIVATION OF EXTRASYNAPTIC NR2B-CONTAINING NMDA RECEPTORS BY HUMAN MONOCYTE-DERIVED MACROPHAGES: IMPLICATIONS FOR PATHOGENESIS OF HIV-1-ASSOCIATED NEUROCOGNITIVE DISORDERS.**. H Xiong[1], JM Yang[1], D Hu[1], J Liu[1]; [1]Dept. of Pharmacology and Exp. Neuroscience, University of Nebraska Medical Center, Omaha, NE 68198-5880.

N-methyl-D-aspartate (NMDA) receptors (NMDARs) play a crucial role in both physiology and pathophysiology. Emerging evidence indicate that the location of NMDARs makes the key difference: survival-promoting signals derive from synaptic NMDARs, which consist of predominant NR2A-containing NMDARs, whereas a cell-death signal comes from extrasynaptic NMDARs, which contain mostly NR2BRs. We hypothesize that HIV1-infected macrophages induce neuronal injury via extrasynaptic NR2BRs. To test this hypothesis, we studied effects of immune-activated, human monocyte-derived macrophages (MDM)-conditioned media (MCM+) on NMDAR-

mediated excitatory postsynaptic currents (EPSCNMDAR) in the CA1 region of rat hippocampal slices. Bath application of MCM+ increased the amplitude of EPSCNMDAR in a concentration dependent manner. In contrast, non-activated MCM had no apparent effect. The MCM(+)-induced increase of EPSCNMDAR was mediated predominantly via NR2BRs. Using MK801 to block synaptic NMDARs, we demonstrated that MCM+ activates extrasynaptic NR2BRs. Biological significance of MCM+ activation of NR2BRs was demonstrated by experimental results that addition of MCM+ to neuronal cultures produced significant neuronal injury and decreased neuronal viability. This MCM(+)-induced cell injury was blocked by an NR2BR antagonist, suggesting that MCM+ produces neuronal injury via extrasynaptic NR2BRs. Our results provide not only novel insights into the mechanisms underlying the pathogenesis of neurodegenerative disorder, but also a new target for the development of therapeutic strategies. Supported by NIH NINDS R01 2NS041862

T-33 **NATURAL KILLER T CELLS INHIBIT HEPATITIS C VIRUS REPLICATION IN HUMAN HEPATOCYTES**. L Ye[1], X Wang[1], SH Wang[1], YJ Wang[1], LI Song[1], WZ Ho[1]; [1]Division of Allergy & Immunology, The Children's Hospital of Philadelphia, Philadelphia, PA 19104-0000, [2]Department of Pediatrics, University of Pennsylvania School of Medicine, Philadelphia, PA 19104-0000.

Natural killer T (NKT) cells are a unique subset of lymphocytes that express both natural killer (NK) and T cell markers. The NKT cells are abundant in liver and play an important role in defense against viral infection. However, the role of NKT cells in control of HCV infection is largely unknown. We investigated whether NKT cells are capable of inhibiting HCV replication in human hepatocytes. Supernatants (SN) collected from primary NKT cell cultures inhibited HCV JFH1 replication in both primary human hepatocytes and hepatoma cell line (Huh7.5.1). When NKT cells were co-cultured with the HCV JFH1 infected-hepatic cells, they had no direct cytolytic effect on hepatocytes, but released soluble factor(s) suppressing HCV replication. The antibody to interferon (IFN)-gamma or IFN-gamma receptors could compromise but not abolish completely the NKT cell-mediated anti-HCV activity, suggesting the IFN-gamma is the major factor for NKT cell-mediated anti-HCV activity. The role of IFN-gamma is supported by the observation that NKT SN enhanced expression of a serial of crucial elements of JAK/STAT pathway. NKT SN also enhanced intracellular IFN-alpha/beta expression in human hepatocytes, suggesting a mechanism responsible for NKT cell-mediated anti-HCV activity. In addition, NKT SN treatment inhibited the expression of the liver-specific miRNA122 that is essential for HCV replication and enhanced the expression of miRNA196a that has anti-HCV property. These findings provide direct evidence that NKT cells have a crucial role in host innate immunity against HCV infection. Supported by NIH DA 12815 and DA 16022

T-34 **EXPRESSION AND REGULATION OF ANTIVIRAL PROTEIN APOBEC3G IN HUMAN NEURONAL CELLS**. L Zhou[1], YJ Wang[1], DL Kolson[2], L Song[1], X Wang[1], L Ye[1], T Zhang[1], H Zhang[3], WZ Ho[1]; [1]Division of Allergy and Immunology/Pediatrics, The Children's Hospital of Philadelphia, Philadelphia, PA 19104-0000, [2]Department of Neurology, University of Pennsylvania School of Medicine, Philadelphia, PA 19104-0000, [3]Division of Infectious Disease/Medicine Department, Thomas Jefferson University , Philadelphia, PA 19107-0000.

Apolipoprotein B mRNA-editing enzyme, catalytic polypeptide-like 3G (APOBEC3G) has recently been identified as a potent antiviral protein. Here, we examined the expression and regulation of APOBEC3G in human brain tissues and the cells of central neurons system (CNS). Similar to the immune cells, human brain tissue and the CNS cells expressed APOBEC3G at both mRNA and protein levels. The expression of APOBEC3G could be up-regulated in human neuronal cells (NT2-N) by inflammatory cytokines (interferons, interleukin-1, and tumor necrosis factor). Other cytokines (interleukin-2, interleukin-4, interleukin-6, regulated upon activation, normal T-cell expressed and secreted, macrophage inflammatory protein-1 alpha and beta), however, had little impact on the expression of APOBEC3G. In addition, cytokine-enriched supernatants from lipopolysaccharide (LPS)-stimulated macrophage cultures induced APOBEC3G expression in NT2-N cells. These findings provide direct evidence that there is intraneuronal expression and regulation of APOBEC3G, which may be a crucial self-defense mechanism in neuronal protection in the CNS. Supported by NIH (DA12815 and DA22177)

T-35 **DIFFERENTIAL INHIBITORY EFFECTS OF HIV-1 TAT PROTEIN ON [3H]DOPAMINE UPTAKE AND [3H]WIN 35,428 BINDING SITES IN RAT STRIATAL SYNAPTOSOMES**. J Zhu[1], CF Mactutus[1], RM Booze[1]; [1]Univ. of South Carolina, Dept. of Psychology, Columbia, SC 29208-0000.

HIV-1 Tat protein plays a key role in pathogenesis of HIV associated neurological impairments. Clinical studies have shown that dopamine (DA) transporter (DAT) activity was strikingly decreased in patients with HAD. However, little is known about the potential mechanistic interactions between Tat with DAT proteins. Rat striatal synaptosomes were incubated with Tat, and then [3H]DA uptake and [3H]WIN 35,428 binding were measured. Results revealed that Tat (70 nM) decreased [3H]DA uptake at 5-60 min, and a maximal decrease was observed at 15 min. Tat inhibited [3H]DA uptake with an affinity (IC50, 3.5μM), while mutant Tat?31-61 did not inhibit [3H]DA uptake in an range of concentration (0.1nM □ 10μM). Kinetic analyses of [3H]DA uptake in the presence of Tat (1 μM) revealed a decrease (25%) in the maximal velocity (Vmax) with no change in Km, compared to control. These results indicate a noncompetitive

interaction of Tat with DA uptake sites. Further, Tat-induced decrease in Vmax of [3H]DA uptake returned to control level after removal of Tat from synaptosomes, indicating the inhibitory effect of Tat on DA uptake is reversible. Also, Tat inhibited [3H]WIN 35,428 binding with IC50 values of 1.2 µM. However, saturation binding studies with [3H]WIN 35,428 showed that Tat (1 µM) decreased the maximal binding sites and increased the Kd compared to control, suggesting Tat-mediated uncompetitive inhibition of [3H]WIN 35,428 binding sites. Thus, the current results suggest a complex interaction of Tat with DAT, which could result in neurologic impairments following HIV-1 infection. Supported by DA013712;DA013137;DA009160

Speaker Abstracts

In alphabetical order

SS-1 Do opiates increase the risk of sepsis? A clinical perspective. A Brack[1], HL Rittner[1]; [1]Klinik für Anesthesiology und Intensivmedizin, Charité - Universitätsmedizin Berlin, Berlin, 12200.

Opioids have been shown to increase the risk and severity of sepsis in numerous experimental studies. Experimental data, however, cannot be easily extrapolated to human sepsis since (i) animal models of sepsis do not adequately reflect human disease and often overestimate clinical benefit (1); (ii) animal studies and patient care are fundamentally different regarding important issues such as age, coexisting diseases or concurrent medication. Studies in humans have provided conflicting data regarding the relationship between opioids and sepsis. Interruption of analgosedation decreases duration of ventilation, ICU and hospital stay and even mortality. However these data were not necessarily related to opioid usage and rates of infection were not analysed (2,3). In retrospective studies, morphine consumption correlated with the cumulative risk of infection in burn patients (4) and with the incidence of pneumonia in cardiothoracic surgery (5). In prospective studies, enteral opioid antagonists decreased the risk of pneumonia in ventilated ICU patients (6). In contrast, perioperative morphine treatment suppressed the hypothalamic-pituitary-adrenal axis and decreased the incidence of pneumonia in alcohol dependent patients undergoing aerodigestive surgery (7). Taken together, opioids might increase the risk of infection and sepsis in humans but evidence

thus far is not conclusive and further studies are urgently needed. (1) Rittirsch J Leukoc Biol 2007; 81:137 (2) Kress JP NEJM 2000; 342:1471 (3) Girard TD Lancet 2008: 371:126 (4) Schwacha MG Am J Surg 2006; 192:82 (5) El Solh AA Resp Med 2006; 100:729 (6) Meissner W Crit Care Med 2003; 31: 776 (7) Spies C Am J Respir Crit Care Med 2006; 174: 408

SS-2 ETHANOL MODULATION OF ASTROGLIAL CHEMOKINE EXPRESSION: IMPLICATIONS IN NEUROAIDS. RL Davis[1]; [1]Department of Pharmacology/Physiology, Oklahoma State University Ctr for Health Sciences, Tulsa, OK 74107-0000.

Alcohol abuse is prevalent among individuals infected with HIV-1. Increasing evidence suggests that alcohol and HIV-1 infection synergistically contribute to neuropathogenesis. Chemokines such as CCL2 and CXCL10 are elevated in HIV-1 infection, but the neurotoxic and neuroprotective contributions of these chemokines in HIV-1 associated neuropathogenesis remain to be fully elucidated. Additionally, ethanol exposure modulates expression of these chemokines in the brain. We are therefore particularly interested in identifying the effects of ethanol on proinflammatory-induced chemokine expression in human astroglial cells. Using an in vitro model of neuroinflammation, we provide insights on the effects of ethanol on proinflammatory-induced CCL2 and CXCL10 expression in human astroglial cells. Depending on the proinflammatory stimulus (TNF?, IFN? or HIV-1 Tat1-72) 3 d ethanol exposure can produce differential effects on CXCL10 expression. For instance, initial findings indicate that ethanol inhibits IFN?- or IFN? + HIV-1 Tat1-72 stimulated-CXCL10 expression, but has little effect on TNF?-induced CXCL10 expression. Ethanol effects on chemokine expression also seem to vary depending on the duration of ethanol exposure and length of ethanol withdrawal. Furthermore, ethanol appears to differentially affect CCL2 and CXCL10 expression in human astroglia. Together, these findings may provide insights into ethanol effects on neuroinflammatory associated pathologies, including HIV-associated dementia. Supported by NIH AA014955

SS-3 A NEW ANTIOXIDANT PREVENTS TOXICITY OF HIV PROTEINS WITH METHAMPHETAMINE. N Ercal[1], W Banks[2], L Abraham[1], T Otamis price[2], X Zhang[1]; [1]Biochemistry, Missouri University of Science & Technology, Rolla, MO 65409-0000, [2]School of Medicine, Saint Louis University, St. Louis, MO 63106-0000.

A major complication of HIV-1 infection is the development of HIV-1-associated dementia (HAD). The mechanisms behind HAD are not yet known. Certain studies have indicated that the HIV-1 envelope glycoprotein (gp120) and transregulatory protein (Tat) play a role in the development of HAD. In particular, these proteins affect the integrity of the blood brain barrier (BBB) by crossing the BBB, altering BBB transporters, and

disrupting the BBB. Our studies show that these proteins also induce oxidative stress in RBE4 cells, an in vitro BBB model. We determined that gp120 and Tat induced oxidative stress in RBE4 cells by measuring selected oxidative stress parameters. Subsequently, the levels of glutathione (GSH), the principal intracellular thiol responsible for maintaining the oxidative balance in cells, significantly decreased, indicating that the cells were undergoing oxidative stress. Antioxidants are becoming increasingly popular in oxidative stress-related disorders and hold promise as therapeutic agents. We have also found that N-acetylcysteine amide (NACA), a new thiol antioxidant, significantly increased the levels of GSH in gp120 and Tat-exposed RBE4 cells. Furthermore, many AIDs/HIV-positive patients use addictive drugs, including methamphetamines (METH). Since METH induces oxidative stress as well, drug abusing patients might be at risk of a synergistic effect and increased damage. Therefore, whether the potent antioxidant NACA protects the BBB from gp120, Tat, or METH (alone and/or in combination) will be discussed. Supported by NIH 1 R15 DA023409-01A2

SS-4 NEW DIRECTIONS AND INITIATIVES AT NIDA. L Erinoff[1]; [1]AIDS Research Program, National Institute on Drug Abuse, Bethesda, MD 20892-0000.

This session will provide an overview of the process by which NIDA develops new initiatives. It will also familiarize the audience with the AIDS Research Program (ARP) Web site. The ARP Web site is updated frequently and lists current AIDS Funding Opportunity Announcements (FOAs)□RFAs, PAs, and PASs. The site also includes AIDS priorities; NIDA program contacts; meeting summaries, links to OAR, NIAID and CDC; and the definition tool for AIDS grants. Dr. Jag Khalsa will present an overview of the Medical Consequences HIV/AIDS research program. This includes research on drug-drug interactions between drugs of abuse, drugs to treat addiction and mental disorders (anxiety and depression, etc.), and antiretrovirals (ARVs) and agents used to treat coinfections such as HCV and TB as well as research on HCV/HIV dual infection. Dr. Diane Lawrence will describe the basic science HIV/AIDS research program which includes effects of drugs of abuse on AIDS and neuroAIDS pathogenesis in various model systems. Also, she will describe current research priorities related to host genetics, proteomics, and glial-neuronal interactions as they relate to HIV/AIDS and drug abuse.

SS-5 NOVEL INDUSTRIAL-ACADEMIC PARTNERSHIPS IN DEVELOPING CELL-BASED NANOFROMULATIONS FOR NEURODEGENERATIVE DISEASES. HE Gendelman[1], BE Rabinow[2]; [1]Department of Pharmacology and Exp. Neuroscience, University of Nebraska Medical Center, Omaha, NE 68198-5880, [2]., Baxter Healthcare Corp., Round Lake, IL 60073-0000.

The development of novel drug delivery systems (DDS) that can specifically target the diseased subregions of the central nervous system (CNS) will profoundly impact the ways drugs are administered. To this end our laboratories, in conjunction with Baxter Healthcare, have developed cell-based nanoparticle-drug formulations for treatment of brain tumors, neuroAIDS, and Parkinson's disease. This lecture will review the synthesis and actions (anti-inflammatory, anti-retroviral, and anti-tumor) for nanoparticle drugs which are absorbed onto particle surfaces, entrapped inside a polymer/lipid, or dissolved within a particle matrix. Solid lipid nanoparticles and dendrimers polymeric vesicles were used to develop treatment paradigms for brain tumors and HIV-1 encephalitis. A macrophage delivery system containing nanoparticles as a DDS was shown to induce a measurable treatment impact. Medical imaging demonstrated disease specificity of the nanoformulation delivery vehicle.

SS-6 SPHINGOLIPID, REDOX AND INFLAMMATORY PERSPECTIVES ON NEURODEGENERATION IN AIDS. NJ Haughey[1]; [1]Neuroimmunology and Neurological Infections, The Johns Hopkins University School of Medicine, Baltimore, MD 21287-0000.

Neurological complications continue to compromise the quality of life for many individuals infected with HIV. A wealth of evidence suggests that multiple signaling pathways are perturbed in the HIV-infected brain that result in cytokine imbalance, mitochondrial dysfunction, oxidative stress, channelopathies and abnormal lipid metabolism (among others). In this milieu, sphingolipids have recently emerged as important bioactive molecules critically involved in survival, neurite outgrowth and synaptic plasticity. Several key regulators of sphingolipid metabolism including cytokines and redox balance are deregulated in HIV dementia, suggesting that these pathways may converge to disrupt sphingolipid balance. While the exact molecular mechanisms by which sphingolipids modulate neuronal function are just beginning to be understood, recent evidence suggests important roles for modulating synaptic strength. Rapid and transient shifts in the sphingolipid content of postsynaptic membranes can regulate glutamate receptor trafficking and synaptic plasticity, while slow and long lasting changes can □trap□ receptors into death-signaling complexes. Several neurotoxins, including the HIV-proteins gp120 and Tat (by separate mechanisms) perturb sphingolipid metabolism and cluster NMDA receptors into microdomains where focal calcium bursts are sufficiently elevated to trigger apoptotic cascades. Normalization of sphingolipid content rescues neurons from apoptosis, suggesting important roles for lipid metabolism in neuronal dysfunction associated with HIV dementia. Supported by MH077542, AA017408, AG023471

SS-7 CANNABINOID SUPPRESSION OF THE T HELPER CELL POLARIZING FUNCTION OF DENDRITIC CELLS. T Klein[1], C Newton[1], L Lu[2]; [1]Molecular Medicine, College of Medicine, University of South Florida, Tampa, FL 33612-0000, [2]Cellular Immunology Section, NIH, NIAID, Bethesda, MD 20892-0000.

Marijuana cannabinoids (CBN) and other neuroimmune modulators suppress Th1 immunity in animal and human studies resulting in distinctive drug-induced profiles of innate and adaptive cytokines. We reported that Legionella pneumophila (Lp) infected mice co-treated with THC had suppressed blood levels of the major Th1 polarizing cytokine, IL-12, and that this effect involved cannabinoid receptors. This suggested that a target of THC might be the dendritic cell (DC) which is a major producer of IL-12. To study this further, we purified DCs from mouse bone marrow and cultured them with Lp and various cannabinoid drugs. Legionella infection was shown to robustly increase DC production of IL-12, IL-6, and TNF alpha and this was suppressed by CBN treatment. Activation marker expression in response to Lp on DCs such as MHC II, CD86, CD40, and the Notch ligand, Delta4, was also suppressed following CBN treatment suggesting CBN directly suppressed DC Th1 polarization. Suppression of Th1 polarization was also shown functionally in that DCs treated with CBN in culture and loaded with Lp failed to induce the development of splenic Th1 cells when passively transferred into naïve recipient mice. Finally, CB1 and CB2 cannabinoid receptors were shown to be involved in the drug effects on DCs. These results suggest that CBN-based drugs might be of value in treating chronic inflammatory diseases with a Th1 immune basis. However, use of these drugs might also enhance Th2 immunity resulting in heightened IgE-mediated diseases. Supported by DA03646 and DA019824, AI45169

SS-8 ALCOHOL AND NEUROAIDS: NEUROIMAGING AND COGNITION. DJ Meyerhoff[1]; [1]Center for Imaging of Neurodegenerative Diseases, VA Medical Center and University of California, San Francisco, CA 94121-0000.

Both heavy alcohol use and HIV infection are associated with increased risk of structural and metabolic brain abnormalities and neuropsychological deficits. Here I will focus on our magnetic resonance neuroimaging and cognitive findings in community-residing HIV+ and HIV- light and heavy drinkers, under consideration of the effects of immune suppression and viral load. HIV-associated brain atrophy is still apparent with effective antiretroviral therapy (ART), exacerbated by heavy drinking. Our longitudinal studies show that brain tissue loss and metabolite injury is ongoing, even when viral load is suppressed. In addition, active heavy alcohol use and HIV infection have additive or synergistic adverse effects on cognitive function, which may mediate health-related behaviors in HIV disease. Thus, HIV associated-brain injury continues despite effective viral suppression and appears worse with heavy alcohol consumption. Supported by P01 AA11493, R01 AA10788

SS-9 HIV-1 CNS INFECTION: BLOOD BRAIN BARRIER (BBB) INJURY AND CO-MORBIDITY FACTORS. Y Persidsky[1]; [1]Path/Microbiol & Pharmacol/Experimental Neurosci, University of Nebraska Medical Center, Omaha, NE 68198-5215.

The major components of the BBB are brain microvascular endothelial cells (BMVEC) at the interface between the blood and the brain. Our works proved the association of BBB injury with the intensity of monocyte infiltration in human brain tissue with HIV-1 encephalitis (HIVE) and in an animal model for HIVE. We showed that HIV-1 infected monocytes interactions with BMVEC triggered RhoA and Rho kinase (RhoK) activation, phosphorylated tight junction (TJ) proteins, and diminished BBB integrity suggesting that RhoA/RhoK activation in BMVEC could be an underlying cause of barrier impairment. Next, we identified specific phosphorylation sites of cytoplasmic domains of TJ proteins by the RhoK, and specific antibodies developed for these sites demonstrated enhanced staining of BMVEC in the mouse model for HIVE and human HIVE. While BBB dysfunction was recently documented after methamphetamine (METH) exposure in vivo, its underlying mechanisms are currently unknown. Our experiments indicated that METH exposure caused generation of reactive oxygen species in human BMVEC, decreased BBB integrity in vitro, diminished expression of TJ proteins, enhanced adhesion and migration of monocytes across endothelial monolayers, and activated RhoA and Rac1 GTPases in BMVEC, the mechanisms previously implicated in the BBB injury during HIVE. Therefore, METH abuse can be a co-morbidity factor via its effects on the BBB. Identification of underlying mechanisms for BBB injury will lead to new therapeutic interventions protecting the BBB and preventing neurodegeneration. Supported by NIH/NIMH RO1 MH6515, UNMC developmental fund

SS-10 SIGNALING PATHWAYS IN HIV-1 TAT-INDUCED CEREBROVASCULAR PATHOLOGY. M Toborek[1]; [1]Department of Neurosurgery, University of Kentucky Medical Center, Lexington, KY 40536-0000.

The blood-brain barrier (BBB) is the critical structure for preventing HIV trafficking into the brain. Specific HIV proteins, such as Tat protein, can contribute to the dysfunction of tight junctions at the BBB and HIV entry into the brain. Tat is released by HIV-infected cells and can interact with a variety of cell surface receptors activating several signal transduction pathways, including those localized in caveolae. Therefore, our research focused on the mechanisms of Tat-induced caveolae-associated Ras signaling at the level of the BBB. Treatment with Tat activated the Ras pathway in human brain microvascular endothelial cells (HBMEC). In addition, exposure to HIV or Tat diminished the expression of several tight junction proteins in the caveolar fraction of HBMEC. These effects were effectively protected by pharmacological inhibition of the Ras signaling and by silencing of caveolin-1. HIV-mediated dysregulation of tight junction proteins was also attenuated by overexpression of peroxisome proliferator-

activated receptor (PPAR)? or PPAR? in HBMEC via downregulation of matrix metalloproteinase (MMP) and proteasome activities. The present data indicate the importance of caveolae-associated signaling in the disruption of tight junctions upon Tat or HIV exposure. They also demonstrate that caveolin-1 and PPARs may constitute early and critical modulators that control signaling pathways leading to HIV-1-induced disruption of tight junction proteins. Supported by MH63022, MH072567, and NS39254

SS-11 MODULATION OF CANNABINOID RECEPTOR ACTIVATION AS A NEUROPROTECTIVE STRATEGY. RF Tuma[1], M Zhang[1], BR Martin[2], RK Razdan[3], MW Adler[1], D Ganea[1]; [1]Center for Substance Abuse Research, Temple University School of Medicine, Philadelphia, PA 19140-0000, [2]Department of Pharmacology and Toxicology,, Virginia Commonwealth University School of Medicin, Richmond, VA 23298-0000, [3]Research and Developmet, Organix Inc., Woburn, MA 01811-0000.

Studies conducted in our laboratory demonstrated that CB2 activation attenuated the progression of disease in an EAE mouse model of multiple sclerosis. In these studies we were able to demonstrate that treatment with a CB2 agonist attenuated leukocyte rolling and adhesion in the pial microvessels of mice during EAE. Since white blood cells invasion is also a contributor to the exacerbation of reperfusion injury to the brain following ischemia, we investigated the potential for selective CB2 activation to attenuate damage after ischemia in a mouse model of stroke. In these studies we provided evidence that selective CB2 activation decreased white cell rolling and adhesion to pial microvessels during reperfusion, and that this attenuation of rolling and adhesion was associated with a reduction in infarct size and an improvement in motor function. While activation of the CB2 receptor was found to reduce reperfusion injury, antagonists for the CB1 receptor were also found to have a protective effect. We therefore proceeded to investigate the effect of treating the animals with both a CB2 agonist and a CB1 antagonist. The results of this investigation demonstrated that his combination had a synergistic effect in reducing infarct size following focal ischemia. We have also demonstrated a similar effect in a mouse model of spinal cord contusion injury. Animals treated with either a CB2 agonist or a CB1 antagonist had more rapid and complete return of bladder function and improved motor function. Combining the CB2 agonist and the CB1 antagonist provided greater protection than the Supported by NIH- DA P30 13429, DA 03672, and DA 05488 from the National

SS-12 CHRONIC COCAINE ADMINISTRATION TO SCID MICE WITH HIV ENCEPHALITIS. WR Tyor[2], WC Griffin[2], LD Middaugh[2]; [1]Department of Neurosciences, Medical University of South Carolina, Charleston, SC 29425-0000, [2]Department of Psychiatry, Medical University of South Carolina, Charleston, SC 29425-0000.

Clinical and basic research suggests that cocaine has detrimental effects on HIV encephalitis (HIVE). In this study mice were inoculated into the frontal lobe with either HIV-infected or uninfected macrophages (controls) and then injected intraperitoneally with either cocaine or saline daily. Cocaine (20 mg/kg) was administered to SCID mice with HIVE and controls according to a dose consistent with euphoria and increased activity of the mice. This daily cocaine regimen (except weekends) was perhaps similar to how cocaine-abusing HIV-infected patients may chronically use this drug. The week following intracerebral inoculation of human macrophages cognition was assessed using the Morris Water maze paradigm, while the mice continued to receive cocaine injections. Fatigue was then monitored by assessing motor activity following a 2 minute forced swim. After cognitive and motor testing was completed mice were sacrificed to determine the extent of astrogliosis and microgliosis in the four groups. As previously reported, in comparison to uninfected controls, HIVE mice had increased astrogliosis and microgliosis, cognitive deficits, and recovered more slowly from fatigue. However, even though the cocaine exposure regimen produced activating and long term CNS effects, the drug did not exacerbate cognitive deficits or neuropathological abnormalities noted in HIV-infected SCID mice, contrary to what was anticipated. Supported by WCG supported by T32 DA07288; WRT and LDM supported by NIDA R01 DA11870