

17th SNIP Scientific Conference

Clearwater Beach, FL
April 5-10, 2011

Society on NeuroImmune Pharmacology Administrative Meetings

Tuesday, April 5, 2011

1:00 PM	Opening of Conference Office
3:00 – 4:30 PM	SNIP Executive Committee Meeting
4:30 – 6:30 PM	SNIP Meetings Committee
7:00 – 9:30 PM	SNIP Council Dinner

Wednesday, April 6, 2011

8:30 – 9:00 AM	Awards Committee
9:00 – 10:00 AM	Finance Committee
10:00 – 11:00 AM	Communications Committee
11:00 AM – Noon	Membership Committee
Noon – 1:15 PM	Lunch – on your own
1:15 – 3:00 PM	Council Meeting and Committee Reports
2:00 PM	Conference Office opens

Scientific Sessions

Wednesday, April 6, 2011

3:00 – 6:00 PM	Registration Opens
4:45 – 7:15 PM	Opening Reception

4:45 – 7:15 PM

POSTER SESSION I – Young Investigators Session

Please have ALL posters mounted on poster boards BY 4:45 PM.

Odd numbered posters (W1, W3, etc.) to be presented from 4:45 – 6:00 PM

Even numbered posters (W2, W4, etc.) to be presented from 6:00 – 7:15 PM

Please remove all posters after the session

Poster Titles listed by assigned Poster Board Numbers (To be announced)

7:30 – 9:00 PM

Meet the Mentors Dinner

**Hosted by Sylvia Kiertscher, Ph.D. (David Geffen School of Medicine/UCLA)
Brian Wigdahl, Ph.D. (Drexel University College of Medicine)
and the Young Investigator's Award Committee**

Featured speaker: Larry Robinson, Ph.D., Provost, Seton Hall University

Lecture: "The Value of Mentoring"

*For Young Investigators who are presenting their work at the Conference and who have **Confirmed their Dinner Reservation** with the YITA Awards Committee*

Thursday, April 7, 2011

7:00 – 8:00 AM

Continental Breakfast

Reminder - Put up Posters for Poster Session II

8:00 – 8:15 AM

INTRODUCTION TO THE MEETING

Welcome from the Society on Neuroimmune Pharmacology

8:00 – 8:10

Toby K. Eisenstein, Ph.D. - SNIP President

(Temple University School of Medicine, Philadelphia, PA)

- Acknowledgement of NIH Staff and Officials

- Acknowledgement of Key Sponsors, Committees and Chairs

8:10 – 8:15

Sulie L. Chang, Ph.D. - Chair, SNIP Meetings Committee

(Seton Hall University, South Orange, NJ)

- Conference Overview & Business

8:15 – 9:05 AM

PLENARY LECTURE I: Horace H. Loh, Ph.D. – University of Minnesota

8:15 – 8:20

Introduction by **Toby K. Eisenstein, Ph.D. – SNIP President**

8:20 – 9:05

Lecture: "Receptor Engineering in the Treatment of Pain – Our Search for the Ideal Analgesic"

9:10 – 11:20 AM

SYMPOSIUM I: Neuroimmune Interactions – The New Frontier for Drugs of Abuse and their Endogenous Ligands

Session Co-Chairs: Martin W. Adler, Ph.D. – Temple University School of Medicine, Philadelphia, PA

Toby K. Eisenstein, Ph.D. – Temple University School of Medicine, Philadelphia, PA

- 9:10 – 9:30 **Lecture 1: Toby K. Eisenstein, Ph.D.** – Temple University School of Medicine, Philadelphia, PA
“Neuroimmune Interactions and Drugs of Abuse Come of Age”
- 9:35 – 9:55 AM** **Coffee Break**
- 9:55 – 10:25 **Symposium Lecture: Kevin J. Tracey, Ph.D.** – Director, Feinstein Institute for Medical Research, Manhasset, NY
“The Cholinergic Anti-Inflammatory Pathway and Innate Immunity”
- 10:30 – 10:50 **Lecture 2: Lynn Kirby, Ph.D.** – Temple University School of Medicine
“Chemokines as Neuromodulators”
- 10:55 – 11:15 **Lecture 3: Virginia M. Sanders, Ph.D.** –Ohio State University College of Medicine
“Sympathetic Nervous System and Norepinephrine: Adrenergic Receptor-Mediated Effects on Immune Function”
- 11:20 – 12:20 PM** **Lunch on your own**
- 12:20 – 1:20 PM** **SNIP Annual Business Meeting**
All Society Members are requested to attend and all attendees welcome
- 1:20 – 3:20 PM** **POSTER SESSION II – General Poster Session**
Please have **ALL** posters mounted on poster boards **BY** 1:20 PM.
Odd numbered posters (T1, T3, etc.) to be presented from 1:20 – 2:20 PM
Even numbered posters (T2, T4, etc.) to be presented from 2:20 – 3:20 PM
- Note: Coffee Break at 2:45 – 3:15 PM during the Poster Session**
- Poster Titles listed by assigned Poster Board Numbers (To be announced)**
- Please remember to take down all posters immediately after the session***
- 3:20 – 5:35 PM** **SYMPOSIUM II: Modulation of the Peripheral Immune System by Drugs of Abuse and HIV**
- Session Co-Chairs: Guy A. Cabral, Ph.D.** – Virginia Commonwealth University, School of Medicine, Richmond, VA
Sabita Roy, Ph.D. – University of Minnesota Academic Health Center, Minneapolis, MN
- 3:20 – 3:50 **Symposium Lecture: Satya Dandekar, Ph.D.** – University of California at Davis, Davis, CA
“Gut, Germs and HIV Pathogenesis of Immune and Neurological Disease”
- 3:55 – 4:15 **Lecture 1: Lena Al-Harathi, Ph.D.** – Rush University Medical Center, Chicago, IL
“The Role of T cell Activation and T cell Subset in Anti-HIV Immunity”

- 4:20 – 4:40 **Lecture 2: Tom Molitor, Ph.D.** – University of Minnesota College of Veterinary Medicine, Minneapolis, MN
“Drug Abuse Modulates Maternal Immunity and its Influence on Neonatal Immune Development”
- 4:45 – 5:00 **Lecture 3: Honghong Yao, Ph.D.** – University of Nebraska Medical Center, Omaha, NE
“Cocaine-Mediated Induction of Platelet-Derived Growth Factor: Implication for Increased Vascular Permeability”
- 5:05 – 5:20 **Lecture 4: Li Liu, Ph.D.** – The University of Hong Kong, Hong Kong SAR, China
“The Effect of Tetrahydrocannabinol (THC) on SIVmac251 Infection in Chinese Macaques”
- 5:25 – 5:45 PM** Refreshments and Late Afternoon Appetizers
- 5:45 – 7:00 PM** **Special Session**
Teaching and Learning in Neuroimmune Pharmacology
- Session Co-Chairs:* **Thomas J Rogers, Ph.D.** – Temple University School of Medicine, Philadelphia, PA
Tom Molitor, Ph.D. – University of Minnesota College of Veterinary Medicine, Minneapolis, MN
- Presentation on **“Video Research Coming of Age”** and a Round-Table Discussion of important issues related to teaching in the field of Neuroimmune Pharmacology with:
Shilpa Buch, Ph.D., Guy Cabral, Ph.D., Sulie L. Chang, Ph.D., Yun-Hsiang Chen, Ph.D., Yuh-Fung Chen, Ph.D., Howard E. Gendelman, M.D., Tom Molitor, Ph.D., Thomas Rogers, Ph.D. and Valerie Wojna, M.D.

Friday, April 8, 2011

- 7:00 – 8:00 AM** **Continental Breakfast**
- 8:00 – 11:00 AM** **SYMPOSIUM III: Management of Neuropsychiatric Complications of Infectious Diseases and Substances of Abuse**
Session Co-Chairs: **Jag Khalsa, Ph.D.** – Chief, Medical Consequences Branch, Division of Pharmacotherapies and Medical Consequences of Drug Abuse, NIDA
Michael Roth, M.D. – David Geffen School of Medicine, UCLA, Los Angeles, CA
- 8:00 – 8:20 **Lecture 1: Dave Thomas, Ph.D.** – Johns Hopkins University of School of Medicine, Baltimore, MD
“Natural History and Pathogenesis of HIV and Hepatitis C”
- 8:25 – 8:45 **Lecture 2: Glen Treisman, M.D.** – Johns Hopkins University of School of Medicine, Baltimore, MD
“Management of Neuropsychiatric Complications of Infection (HIV, HCV)”

- 8:50 – 9:10 **Lecture 3: Susanna Naggie, M.D.** – Duke University School of Medicine, Durham, NC
“Investigational Agents for Chronic Viral Infections: HIV and HCV”
- 9:15 – 9:30 AM** **Coffee Break**
- 9:35 – 11:00 AM** **SYMPOSIUM IV: Therapeutic Strategies Targeting Neuroimmune Modulation**
Session Co-Chairs: **Abraham P. Bautista, Ph.D.** – Director, Office of Extramural Activities/ (NIAAA/NIH)
Jeymohan Joseph, Ph.D. – Chief, HIV Pathogenesis, Neuropsychiatry and Treatment Branch/ Division of AIDS Research (NIMH/NIH)
- 9:35 – 9:55 **Lecture 1: Ru-Band Lu, M.D.** National Cheng Kong University, Taiwan
“Genetic Validation in the Subtypes of Alcoholism.”
- 10:00 – 10:20 **Lecture 2: Paul D. Drew, Ph.D.** – University of Arkansas for Medical Sciences
“Role of Neuroimmune Signaling Molecules in the Neuropathology of Fetal Alcohol Spectrum Disorders”
- 10:25 – 10:45 **Lecture 3: Adron Harris, Ph.D.** – University of Texas at Austin, Austin, TX
“Therapeutic Strategies Targeting Neuroimmune Modulation”
- 10:50 – 11:10 **Lecture 4: Jialin C. Zheng, M.D.** – University of Nebraska Medical Center, Omaha, NE
“Neurogenesis, Brain Inflammation and its Links to the Pathogenesis and Potential Therapy of Neurodegenerative Disorders”
- 11:15 – 11:30 PM** **PICK-UP LUNCHES FOR NIH WORKSHOP**
- 11:30 – 1:00 PM** **NIH WORKSHOP:**
Session Co-Chairs: **David Shurtleff, Ph.D.** – Acting Deputy Director (NIDA/NIH)
Jeymohan Joseph, Ph.D. – Chief, HIV Pathogenesis, Neuropsychiatry and Treatment Branch/ Division of AIDS Research (NIMH/NIH)
Abraham P. Bautista, Ph.D. – Director, Office of Extramural Activities / (NIAAA/NIH)
- Participants
David Shurtleff, Ph.D. – Acting Deputy Director (NIDA/NIH)
Jag Khalsa, Ph.D. – Chief, Medical Consequences Branch, Division of Pharmacotherapies and Medical Consequences of Drug Abuse (NIDA/NIH)
Diane M. Lawrence, Ph.D. – Associate Director AIDS Research Program (NIDA/NIH)
Albert Avila, Ph.D. – Program Director, Division of Basic Neuroscience and Behavioral Research (NIDA/NIH)
Woody Lin, M.D., Ph.D. – Health Scientist Administrator, Division of Clinical Neuroscience and Behavioral Research (NIDA/NIH)
Jeymohan Joseph, Ph.D. – Chief, HIV Pathogenesis, Neuropsychiatry and Treatment Branch/ Division of AIDS Research (NIMH/NIH)
Abraham P. Bautista, Ph.D. – Director, Office of Extramural Activities

(NIAAA/NIH)

Kindall Bryant, Ph.D. – Director, Alcohol and HIV/AIDS Research, OD
(NIAAA/NIH)

Ranga Srinivas, Ph.D. – Chief, Extramural Project Review Branch, OEA
(NIAAA/NIH)

Changhai Cui, Ph.D. – Program Director, Division of Neuroscience and Behavior
(NIAAA/NIH)

Eduardo A. Montalvo, Ph.D. – Scientific Review Officer, Center for Scientific
Review (NIH)

1:00 – 2:30 PM

YOUNG INVESTIGATOR'S SYMPOSIUM

Session Co-Chairs: **Albert Avila, Ph.D.** – Program Director, Division of Basic
Neuroscience and Behavioral Research (NIDA/NIH)

Sylvia M. Kiertscher, Ph.D. – David Geffen School of
Medicine at UCLA

Pre-Doctoral Presentations:

1:00 – 1:10

Crystal Bethel-Brown – Department of Pharmacology and Experimental
Neuroscience, University of Nebraska Medical Center, Omaha, NE
*“HIV-1 Tat Mediated Induction of Platelet-Derived Growth Factor in
Astracytes: Role of Early Growth Response Gene 1”*

1:15 – 1:25

Sharrón L. Manuel - Drexel Institute for Biotechnology & Virology Research,
Drexel University College of Medicine, Philadelphia, PA
*“Dynamics of Dendritic Cells and T Cells in HTLV-1-Associated
Neuroinflammatory Disease: Implications in Immunomodulatory Therapies
and Diagnostic Tools”*

1:30 – 1:40

Ankit Shah – Division of Pharmacology and Toxicology, University of
Missouri-Kansas City, Kansas City, MO
*“HIV-1 Glycoprotein 120 Induces the Pro-Inflammatory Cytokine IL-6 via the
NF-Kappa-B Pathway and Methamphetamine Can Synergistically Potentiate
gp120-Mediated IL-6 Induction”*

Post-Doctoral Presentations:

1:45 – 1:55

Ming D. Duan, Ph.D. - Department of Pharmacology and Experimental
Neuroscience, University of Nebraska Medical Center, Omaha, NE
*“Cocaine Increases Platelet-Derived Growth Factor Expression in Human
Brain Microvessel Endothelial Cells Through Notch-1 Signaling”*

2:00 – 2:10

Lei Chen, Ph.D. - Department of Neurosurgery, University of Kentucky,
Lexington, KY
“Autophagy is Involved in the Neurovascular Toxicity of Nanoalumina”

2:15 – 2:25

Shinsuke Nakagawa, Ph.D. - Department of Neurosurgery, University of
Kentucky, Lexington, KY
*“Human Immunodeficiency Virus Type 1 (HIV-1) Infects Human Brain
Pericytes In Vitro”*

2:30 PM

FREE TIME – Enjoy the Beach!

YES! – THE AFTERNOON AND EVENING ARE FREE

7:30 – 9:30 PM

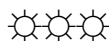
Journal of Neuroimmune Pharmacology Editorial Board Meeting

Saturday, April 9, 2011

- 7:00 - 8:15 AM** **Continental Breakfast**
- 8:15 – 9:00 AM** **PLENARY LECTURE II: Steve Nelson, M.D.** – Dean, LSU School of Medicine, New Orleans, LA
- 8:15 – 8:20 Introduction by **Sulie L. Chang, Ph.D.** - Chair, SNIP Meetings Committee
- 8:20 – 9:00 Lecture: *“Alcohol, Immunosuppression and HIV”*
- 9:05 – 11:10 AM** **SYMPOSIUM V: Cocaine and HIV-1 Interplay: Molecular Mechanisms of Action and Addiction**
- Session Co-Chairs:* **Norman Haughey, Ph.D.**-- Johns Hopkins University School of Medicine, Baltimore, MD
- Michal Toborek, M.D., Ph.D.** – University of Kentucky School of Medicine, Lexington, KY
- 9:05 – 9:35 **Symposium Lecture: Tsung-Ping Su, Ph.D.** – Chief, Cellular Pathobiology Section, Cellular Neurobiology Research Branch, NIDA/NIH
- “Molecular Chaperone and Interorganelle Signaling in Diseases”*
- 9:40 – 10:00 **Lecture 1: John Q. Wang, Ph.D.** –University of Missouri-Kansas City School of Medicine, Kansas City, MO
- “Molecular Neurobiology of Drug Addiction: Role of NMDA Receptors”*
- 10:05 – 10:20 AM** **Coffee Break**
- 10:20 – 10:40 **Lecture 2: Gayle C. Baldwin, Ph.D.** – David Geffen School of Medicine at UCLA, Los Angeles, CA
- “Defining Mechanisms of Cocaine and HIV Co-Morbidity In Vivo: The Potential and Pitfalls of Mouse/Human Chimera Models”*
- 10:45 – 11:05 **Lecture 3: Avi Nath, M.D.** - Johns Hopkins University School of Medicine, Baltimore, MD
- “Fulminant Encephalopathy in HIV-infected Cocaine Abusers”*
- 11:10 – 12:00 Noon** **“Bill” Narayan Lecture: Shilpa Buch, Ph.D.** – University of Nebraska Medical Center, Omaha, NE
- 11:10 – 11:20 Introduction by **David Volsky, Ph.D.** – Columbia University, New York, NY
- 11:20 – 12:00 Lecture: *“HIV Infection and Cocaine Abuse Go Hand in HAND”*
- Noon – 1:00 PM** **Lunch on your own**
- 1:00 – 2:50 PM** **SYMPOSIUM VI: HIV-Associated Neurocognitive Disorders (HAND) and Drug Abuse**
- Session Co-Chairs:* **Linda Chang, M.D.** – University of Hawaii, Honolulu, HI
- Mahendra Kumar, Ph.D.** – Miller School of Medicine, University of Miami, Miami, FL

- 1:00 – 1:30 **Symposium Lecture: Bob Heaton, Ph.D.** – University of California San Diego, San Diego, CA
“Prevalence and HIV Disease Correlates of HAND in the Pre-CART and CART Eras ”
- 1:35 – 1:55 **Lecture 1: Eileen Martin, Ph.D.** – University of Illinois, Chicago, IL
“NeuroAIDS and Substance Use Disorders”
- 2:00 – 2:20 **Lecture 2: Marilou Andres, Ph.D.** University of Hawaii at Manoa
“Effects of APOE-epsilon4 Allele on Brain Function and Structures in HIV Patients”
- 2:25 – 2:45 **Lecture 3: Valerie Wojna, M.D.** – University of Puerto Rico, Medical Sciences
“Challenges in the Diagnosis of HAND in a Hispanic Cohort of HIV-seropositive Women”
- 2:50 – 3:05 PM Coffee Break**
- 3:05 – 5:20 PM SYMPOSIUM VII: The Consequences of Substance Abuse and HIV on Stem Cell Biology**
- Session Co-Chairs:* **Changhai Cui, Ph.D.** – Program Director, Division of Neuroscience and Behavior (NIAAA/NIH)
Kurt Hauser, Ph.D. – Virginia Commonwealth University, School of Medicine, Richmond, VA
- 3:05 – 3:35 **Symposium Lecture: Pamela Knapp, Ph.D.** – Virginia Commonwealth University, School of Medicine, Richmond, VA.
“Stage Specific Effects of Opiates and HIV on the Differentiation and Function of CNS Progenitors”
- 3:40 – 4:00 **Lecture 1: Amelia Eisch, Ph.D.** – UT Southwestern Medical Center at Dallas
“Adult Hippocampal Neurogenesis and Opiates: Implications for Addiction”
- 4:05 – 4:25 **Lecture 2: Ping Zhang, Ph.D.** – Louisiana State University Medical Center at New Orleans, New Orleans, LA
“The Effects of Excessive Alcohol Consumption on the Development of Myelosuppression During SIV Infection”
- 4:30 – 4:50 **Lecture 3: Pankaj Seth, Ph.D.** – National Brain Research Centre, India
“Neuron-Glia Crosstalk in HIV-1 Neuropathogenesis”
- 6:45 – 10:00 PM EVENING BANQUET AND AWARDS CEREMONY**
- Hosted by **Guy A. Cabral, Ph.D.** - incoming SNIP President
- Special Dinner Presentation: **Timothy Yeatman, M.D.** – Moffitt Cancer Center, University of South Florida & Chief Scientific Officer, M2Gen
“The M2Gen Approach to Personalized Medicine and Drug Development”

Meeting Adjourned!



Sunday, April 10, 2011 – Departure Day

INVITED SPEAKER ABSTRACTS

(in alphabetical order by presenting author)

The Role of T Cell Activation and a T Cell Subset in Anti-HIV Immunity. *L Al-Harhi*¹; ¹Department of Immunology/Microbiology, Rush University Medical Center, Chicago, IL 60612.

CD8⁺ T cells contain a subset that expresses CD4 dimly on their surface (CD4dim/CD8bright T cells) and constitute 3% of peripheral CD8⁺ T cells of healthy individuals. In vitro, CD4dim/CD8bright T cells are induced in response to T cell activation. Ex vivo, percent expression of CD4dim/CD8bright T cells is 5-fold higher among HIV long-term nonprogressors than HIV seronegative and chronically HIV infected donors. CD4dim/CD8bright T cells exhibited greater than 55% of CD8⁺ T cell antigen recognition and effector response to HIV, which were CD4⁻ and MHC-II⁻ dependent. β -catenin expression is higher in CD4dim/CD8bright T cells than their CD4-CD8⁺ T cell counterpart and mediates CD4 and Bcl-xL expression in this subset. We have shown that β -catenin signaling inhibits HIV replication suggesting that endogenous b-catenin expression in CD4dim/CD8bright T cells may serve as an anti-viral factor inhibiting HIV replication and/or mediating protection against apoptosis. Finally, we demonstrate that a common psychostimulant, methamphetamine, inhibits b-catenin signaling suggesting that drug use may impact the generation and/or persistence of CD4dim/CD8bright T cells. Collectively, these data demonstrate that CD4dim/CD8bright T cells designate an enriched subpopulation of anti-HIV CD8⁺ T cells and that biologic or exogenous factors (drugs of abuse) that down regulate β -catenin signaling may adversely affect the generation and/or persistence of this population. Supported by RO1 NS060632, R21 A1077329, R03 DA026723-01, and D-CFAR P30AI082151.

Effects of APOE- ϵ 4 Allele on Brain Function and Structure in HIV Patients. *M Andres*¹, *T Ernst*², *S Sadino*², *C Jiang*², *H Nakama*³, *S Munsaka*², *L Chang*²; ¹Pacific Biosciences Research Center, University of Hawaii at Manoa, Honolulu, HI 96822, ²Department of Medicine, John A. Burns School of Medicine, University of Hawaii at Manoa, Honolulu, HI 96813, ³Department of Psychiatry, John A. Burns School of Medicine, University of Hawaii at Manoa, Honolulu, HI 96813.

Carrying the APOE4 (ϵ 4) allele is a risk factor for Alzheimer's disease, and may also play a role in promoting HIV-associated neurocognitive disorder (HAND). Recent studies demonstrated that homozygosity (but not heterozygosity) for the ϵ 4 allele accelerated the progression of the HIV Disease. While several studies investigated the effects of the APOE4 allele on cognitive function in HIV patients, none have evaluated the effects of the ϵ 4 allele on the brain structure of HIV-infected individuals. Therefore, we genotyped a total of 139 subjects (70 seronegative (SN) controls and 69 HIV+ subjects) and used MR morphometry to assess brain volumes. We found that younger (<50 years) but not older (\geq 50 years) HIV+ subjects who are ϵ 4 carriers have brain atrophy in many subcortical regions compared to younger (<50 years) HIV+ subjects without the ϵ 4 allele. Conversely, among SN controls, this brain atrophy occurred only in older SN subjects with the ϵ 4 allele. HIV+ subjects carrying the ϵ 4 allele also performed worse on cognitive tests than HIV+ subjects without the ϵ 4 allele and SN controls (with and without the ϵ 4 allele). Among HIV+ individuals, those with the ϵ 4 allele had higher levels of CSF APOE and also performed worse on the HIV Dementia scales and had lower Global Cognitive Scores than those without the ϵ 4 allele. Together, these findings suggest that the APOE4 allele and the levels of encoded APOE proteins negatively impact brain function and structure and may facilitate earlier onset of neurodegeneration in the setting of HIV. Supported by 2R01MH61427, 5R25MH080661 2K24DA016170, 1R24DA027318, and 1U54-NS056883.

Defining Mechanisms of Cocaine and HIV Co-Morbidity In Vivo: The Potential and Pitfalls of Mouse/Human Chimera Models. *GC Baldwin*¹, *J Zhuo*¹, *KM Whittaker*¹, *D Vatakis*¹, *MD Roth*¹, *SM Kiertscher*¹; ¹Department of Medicine, David Geffen School of Medicine at UCLA, Los Angeles, CA 90095.

Mouse/human chimera models, in which human cells or tissues are transplanted into immunodeficient strains of mice, have enabled researchers to model disease mechanisms and to characterize the role of potential co-factors relevant to HIV pathogenesis. Humanized mouse models include 1) those transplanted with human stem cells alone or in combination with fetal tissues, which result in a slower but more extensive immune reconstitution and 2) those transplanted with fully differentiated human peripheral blood leukocytes (PBL), which lead to a more rapid but targeted immune reconstitution. We have utilized both types of chimeric models to assess the impact of acute cocaine exposure on HIV replication in vivo,

and to study broader issues relevant to the mechanisms underlying the co-morbidity resulting from the combination of HIV and cocaine. Using conventional SCID, as well as NOD-SCID/IL2 γ null mice as recipients for human PBL, we have shown that acute cocaine exposure significantly enhances HIV infection. We are currently utilizing the huPBL-NOD/SCID model to assess underlying mechanisms, including the impact of cocaine on the induction of Ag-specific responses. To study the impact of chronic cocaine and HIV co-exposure in the periphery and CNS, we are utilizing two chimeric models: NOD-SCID/IL2 γ null mice (transplanted with human CD34+ cord blood stem cells), and BLT mice (transplanted with fetal liver-derived CD34+ stem cells in combination with a fetal liver/thymus implant). Although a number of practical limitations still exist, mouse/human chimera models have made tremendous progress since their inception and are useful tools for understanding the mechanisms of human disease. Supported by NIH/NIDA R01DA023386.

HIV-1 Tat Mediated Induction of Platelet-Derived Growth Factor in Astrocytes: Role of Early Growth Response Gene 1. *C Bethel-Brown*¹, H Yao², S Callen², YH Lee³, PK Dash², A Kumar⁴, S Buch²; ¹Molecular and Integrative Physiology, University of Kansas Medical Center, Kansas City, KS 66160, ²Pharmacology and Experimental Neuroscience, University of Nebraska Medical Center, Omaha, NE 68137, ³Institute of Biomedical Science and Technology, Konkuk University, Seoul, Korea 143-701, ⁴Pharmacology, University of Missouri Kansas City, Kansas City, MO 64108.

HIV-associated neurological disorders (HAND) are estimated to affect almost 60% of HIV infected individuals. HIV-encephalitis (HIVE), the pathological correlate of the most severe form of HAND is often characterized by glial activation, cytokine/chemokine dysregulation, and neuronal damage and loss. However, the severity of HIVE correlates better with glial activation rather than viral load. Using the macaque model, it has been demonstrated that simian immunodeficiency virus encephalitis (SIVE) correlates with increased expression of the mitogen platelet-derived growth factor-B (PDGF-B) chain in the brain. The present study was aimed at exploring the role of PDGF-B chain in HIV-associated activation and proliferation of astrocytes. Specifically, the data herein demonstrate that exposure of rat and human astrocytes to the HIV-1 protein, Tat resulted in the induction of PDGF at both the mRNA and protein levels. Furthermore, PDGF-BB induction was regulated by activation of ERK1/2 and JNK signaling pathways and the downstream transcription factor, early growth response 1 (Egr-1). Chromatin immunoprecipitation (ChIP) assays demonstrated binding of Egr-1 to the PDGF-B promoter. Exposure of astrocytes to PDGF-BB, in turn, led to both increased proliferation and release of pro-inflammatory cytokines MCP-1 and IL-1 β . Since astrogliosis is linked to disease severity, understanding its regulation by PDGF-BB could aid in the development of therapeutic intervention strategies for HAND. Supported by MH-068212, DA020392, DA023397, DA024442, and DA0277729.

HIV-1 Infection and Cocaine Go Hand in HAND. *SJ Buch*¹, H Yao¹; ¹Pharmacology, University of Nebraska Medical Center, Omaha, NE 68198.

Cocaine, often abused by HIV-infected patients, has been suggested to worsen HIV-associated neurocognitive disorders (HAND) via unknown mechanisms. Our recent research interest has focused on understanding molecular mechanisms by which cocaine accelerates pathogenesis of HAND by impacting the functioning of various CNS cell types. In this area, we have demonstrated that: 1) In macrophages, cocaine enhances virus replication & activation, 2) In microglia cocaine upregulates the expression of key chemokine MCP-1, leading to increased influx of inflammatory cells in the brain; 3) In neurons cocaine potentiates neurotoxicity mediated by HIV-1 envelope protein gp120; 4) In human brain microvascular endothelial cells, cocaine induces the expression of the adhesion molecule ALCAM as well as the vascular permeant platelet-derived growth factor (PDGF)-B chain, both of which impair blood brain barrier integrity resulting again in augmentation of endothelial permeability as concomitant increased monocyte transmigration; 5) More recently we have also shown that cocaine-mediated targeting of Notch-1 receptor in brain endothelial cells can also result in induction of PDGF-BB. Functional implication of up-regulated PDGF-BB as a vascular permeant was confirmed both in cell culture and in vivo model systems using permeability & transmigration assays. In summary, besides its addictive role in the CNS, cocaine also has the capacity to negatively impact the functioning of almost all the cells of the CNS leading to increased neuroinflammation and thus contributing to the pathogenesis of HAND. Supported by NIDA grants DA020392, DA023397, DA024442, and DA030285.

Autophagy is Involved in the Neurovascular Toxicity of Nanoalumina. *L Chen*¹, B Hennig², M Toborek¹; ¹Neurosurgery, University of Kentucky, Lexington, KY 40503, ²College of Agriculture, University of Kentucky, Lexington, KY 40503.

Engineered nanomaterials (ENMs) have been widely used for various applications; however, their potential toxic effects are not fully understood. The current study focused on blood-brain barrier (BBB) disruption and neurovascular damage induced by treatment with one of the most abundantly manufactured ENMs, nanoalumina. Exposure of cultured human cerebral endothelial cells to nanoalumina (size 8-12 nm) elevated cellular oxidative stress, induced mitochondrial potential collapse, decreased tight junction expression, and cellular viability. Nanoalumina also induced activation of lysosomes and formation of autophagic vacuoles associated with elevated levels of autophagic marker proteins, p62 and LC-3. Delivery of nanoalumina into mouse cerebral circulation using a carotid surgical method altered expression of autophagy-related genes as detected by the brain DNA microarray. In addition, in situ immunostaining of brain slices detected time-dependent increases in LC-3 levels in different cell types of the neurovascular unit. Systemic treatment with nanoalumina elevated also autophagic activity in cerebral vessels, decreased tight junction expression, and elevated BBB permeability. Finally, exposure to nanoalumina increased brain infarct volume in mice subjected to a focal ischemic stroke model. Collectively, our study reveals that autophagy constitutes one of the critical mechanisms involved in nanoalumina-induced neurovascular toxicity in the central nervous system. Supported by American Heart Association Postdoctoral Scholarship, NIH ES 07380, DA027569, MH63022, MH072567, and NS39254.

Gut, Germs and HIV Pathogenesis of Immune and Neurological Disease. *S Dandekar*¹; ¹Department of Medical Microbiology and Immunology, University of California at Davis, Davis, CA 95616.

Gastrointestinal (GI) and neurological abnormalities are common features of progressive HIV infection. Chronic immune activation in HIV infection serves as a correlate of disease progression and contributes to gastroenteropathy and neuropathology. Mechanisms contributing to the chronic immune activation need to be fully defined in order to prevent HIV associated damage of the immune and neuronal functions. The gut associated lymphoid tissue (GALT) is an important site of early host-virus interactions and for establishment of persistent viral reservoir in HIV infected patients and in the simian immunodeficiency virus (SIV) infected rhesus macaque model of AIDS. Rapid onset of CD4+ T cell depletion and epithelial barrier disruption set the stage for the development of stable viral reservoirs and chronic immune activation. The anti-retroviral therapy fails to either eradicate the viral reservoirs or fully restore mucosal and neuronal functions. A better understanding of the impact of HIV infection on the mucosal immune system and its systemic impact will help define the mechanisms of HIV pathogenesis and novel HIV preventive and therapeutic strategies. Supported by NIH.

Role of Neuroimmune Signaling Molecules in the Neuropathology of Fetal Alcohol Spectrum

Disorders. *PD Drew*¹, L Han¹, RR Smith¹, JC Douglas¹, KD Phelan¹, CJ Kane¹; ¹Department of Neurobiology & Developmental Sciences, University of Arkansas for Medical Sciences, Little Rock, AR 72205.

Fetal Alcohol Spectrum Disorders (FASD), which include Fetal Alcohol Syndrome and Alcohol Related Neurodevelopmental Disorder, occur in 1% of children in the U.S. FASD is the leading cause of mental retardation and is characterized by mild to severe cognitive and behavioral defects and neuropathology that persist throughout life. Despite our understanding that fetal alcohol exposure is associated with loss and dysfunction of neurons, the underlying cellular and molecular mechanisms of pathogenesis remain largely unknown. This knowledge is critical to develop intervention strategies. Recent studies are elucidating a new mechanistic paradigm that may allow intervention. This is built on the discovery that glia are direct targets of alcohol in the developing brain and, thus, may play a role in FASD etiology. Using the neonatal rodent model of FASD and primary cell cultures, we have found that alcohol exposure causes significant loss of microglia and neurons. Because normal microglial-neuronal interactions are important to neuron survival, depletion of microglia may limit their neuroprotective capacity during alcohol exposure. Surviving microglia exhibit altered expression of neuroimmune molecules and an activated cell phenotype. Analysis of alcohol effects on intracellular signaling pathways has revealed potential mechanisms by which microglia may contribute to alcohol induced neurodegeneration. Thus, definition of alcohol effects on microglia and neuroimmune processes holds potential for intervention in the

neuropathology associated with fetal alcohol exposure. Supported by NIH-NIAAA AA14645, AA14888, AA18834, AA18839, and AA19108.

Cocaine Increases Platelet-Derived Growth Factor Expression in Human Brain Microvessel Endothelial Cells Through Notch-1 Signaling. *MD Duan*¹, *HY Yao*¹, *SB Buch*¹; ¹Department of Pharmacology and Experimental Neuroscience, University of Nebraska Medical Center, Omaha, NE 68198.

Neuroinflammation associated with advanced HIV-1 infection is often exacerbated in cocaine-abusing, HIV-infected individuals. The underlying mechanisms could, in part, be attributed to the increased impairment of blood brain barrier integrity in the presence of cocaine. Platelet-Derived Growth Factor-BB (PDGF-BB) has been implicated in a number of pathological conditions, specifically attributable to its potent mitogenic effects. Its modulation by drug abuse, however, has received very little attention. In the present study, we demonstrated cocaine-mediated induction of PDGF-BB and Notch1 in human brain microvascular endothelial cells. In this study, we link Notch1 signaling to PDGF-BB, and show that PDGF-BB is a novel immediate Notch target gene. PDGF-BB expression induced by cocaine was abrogated by gamma secretase inhibitor-DAPT. Furthermore, over-expression of intracellular domain of Notch1 significantly increased PDGF-BB expression. Functional implication of up-regulated PDGF-BB as a vascular permeant was confirmed in cell permeability and transmigration assays. In vivo relevance of these findings were further corroborated in cocaine-treated mice that were administered DAPT (i.p. 40mg/kg). Cocaine exposure resulted in increased permeability of the endothelial barrier and this effect was abrogated in mice exposed to DAPT, thus underscoring its role as a vascular permeates. Understanding the regulation of PDGF-BB expression may provide insights into the development of potential therapeutic targets for neuroinflammation associated with HIV infection and drug abuse.

Adult Hippocampal Neurogenesis and Animal Models of Drug Addiction: Implications for Substance Abuse. *AJ Eisch*¹; ¹Psychiatry Department, University of Texas Southwestern Medical Center, Dallas, TX 75390-9070.

Drugs of abuse, like morphine, heroin and cocaine, produce robust changes in the birth and survival of neurons in the postnatal hippocampal subgranular zone (SGZ). This is intriguing since the hippocampus is central to many aspects of the addictive process, including drug-context associations and relapse to drug taking. Also, the functional integration of postnatally-generated neurons into hippocampal circuitry and the role of new neurons in memory formation raise the possibility that decreased adult SGZ neurogenesis may alter hippocampal function in such a way as to maintain addictive behavior or contribute to relapse. We will review our work on the impact of opiates and cocaine on the different stages of postnatal neurogenesis, focusing on recent data exploring whether cells 'born' during opiate exposure have altered dendritic processes and on animal models of morphine self-administration. Understanding the relationship between drugs of abuse and SGZ neurogenesis opens the possibility of understanding brain functions subserved by neurogenesis, such as memory, and also of harnessing neural stem cells for repair of the diseased and injured brain. Supported by National Institute on Drug Abuse, NASA.

Neuroimmune Interactions and Drugs of Abuse Come of Age. *T Eisenstein*¹; ¹Center for Substance Abuse Research, Temple University School of Medicine, Philadelphia, PA 19140.

Neuroimmunology used to be synonymous with the study of inflammation in the nervous system, frequently caused by leukocyte infiltrates with subsequent tissue and neuronal damage. With the discovery that IL-1, a cytokine, was the "endogenous pyrogen" and caused fever, the idea of a neural-immune connection with a physiological basis was born. Among the salient subsequent discoveries were the observations of Wybran, from which he inferred that leukocytes express opioid receptors, providing an example of a circuit in which a neuronal product modulates the immune system. We now have a significant panel of molecules originally discovered in the nervous system or the immune system that have been found to be present in the other system or to target the other system, including Substance P, Vasoactive Intestinal Peptide, epinephrine, acetylcholine, IL-6, and the chemokines. An unpredicted and intriguing finding is that neurons have receptors for chemokines and can secrete them. In the last ten years it has been found that leukocytes are the primary cells in the body that express cannabinoid receptor 2 (CB2), and that CB2 agonists are anti-inflammatory. Some of ligands in these diverse systems have homeostatic functions. Research on drugs of abuse and the immune system has been at the

forefront of the discoveries that are uncovering the intimate interactions between the neural and immune systems. This Symposium will explore some of the novel findings in this important and growing area of neuroimmune interactions. Supported by NIDA P30 DA13429.

Immune Signaling Regulates Alcohol Consumption: Role of Neuroimmune Gene Expression. *A Harris*¹, I Ponomarev¹, Y Blednov¹; ¹Waggoner Center for Alcohol and Addiction Research, University of Texas at Austin, Austin, TX 78712.

Immune/stress response genes are a prominent functional group with differential expression in frontal cortex and VTA of alcoholics in comparison to non-alcoholics. In addition, brain gene expression in mice that are genetically predisposed to alcohol consumption implicates these pro-inflammatory mediators in regulating alcohol intake. We selected six genes [β -2-microglobulin (B2M), cathepsin S (CTSS), cathepsin F (CTSF), interleukin 1 receptor antagonist (IL1RN), CD14 (CD14) and interleukin 6 (IL6)] for behavioral validation. Null mutant mice were tested for ethanol intake in three tests: 24 hr two-bottle choice, limited access two-bottle choice and limited access to one bottle of ethanol. Ethanol consumption and preference were reduced in all the null mutant mice in the 24 hr two-bottle choice test, the test that was the basis for selection of these genes. No major differences were observed in consumption of saccharin in the null mutant mice. Deletion of B2M, CTSS, IL1RN, CD14 and IL6 also reduced ethanol consumption in the limited access two bottle choice test for ethanol intake; with the IL1RN and CTSS null mutants showing reduced intake in all three tests (with some variation between males and females). We next asked if activation of the immune system could promote excessive alcohol consumption. TLR4 signaling (LPS, 1 mg/kg ip, C57/Bl6 mice) promoted alcohol consumption, an effect persisting at least 3 month after a single LPS dose and not associated with general changes in taste perception or palatability. The lack of CD14 (*CD14* knockout mice) as well as IL6 (*IL6* knockout mice) prevented the increase of alcohol intake after LPS. Taken together, this provides compelling evidence that global gene expression analysis can identify novel genetic determinants of complex behavioral traits and suggests a novel role for neuroimmune signaling in regulation of alcohol consumption. Supported by the National Institute on Alcohol Abuse and Alcoholism (AA U01 13520 - INIA Project; and AA06399).

Prevalence and HIV Disease Correlates of HAND in the Pre-CART versus CART Eras. *RK Heaton*¹, D Franklin¹, R Ellis¹, S Letendre², JA McCutchan², I Grant¹; ¹UCSD Department of Psychiatry, University of California, San Diego, La Jolla, CA 92093, ²Department of Medicine, University of California San Diego School of Medicine, La Jolla, CA 92093-0671.

Combination antiretroviral therapy (CART) has greatly reduced the incidence of opportunistic disease and mortality in HIV-infected individuals, but studies of its effect on neurocognitive impairment (NCI) have been mixed. Here we examined NCI in the pre-CART and CART eras in persons at various stages of HIV infection. 857 adults (HIV-, n=179; non-AIDS, n=516; AIDS, n=162) from the pre-CART era (1988-1995) were compared to 937 (HIV-, n=94; non-AIDS, n=336; AIDS, n=506) from the CART era (2000-2007). Treatment era cohorts received comparable, comprehensive neuromedical and neuropsychological evaluations, and similar, rigorous screening to exclude non-HIV CNS comorbidities. Overall, 40% of HIV-infected individuals had NCI in the CART era vs. 33% in the pre-CART era. Analysis by CDC Stages showed a significantly higher rate of NCI for CART era in CDC A only (36% post-CART vs 25% pre-CART, $p = .001$). Infected CART era individuals were more likely to be on ARVs (70% vs. 47%; $p < .0001$). History of severe immunosuppression (low nadir CD4) was the only robust predictor of NCI in both eras. NCI remains prevalent despite CART. Of interest, more CART era non-AIDS cases have NCI than pre-CART. This suggests negative CNS effects of longer survival in a pre-AIDS state during which the brain remains exposed to repeated fluxes in HIV and/or chronic immune stimulation. HNRC supported by a Center Award (MH62512) with the CNS HIV Antiretroviral Therapy Effects Research (CHARTER) study supported by Award N01 MH22005, both from the National Institute of Mental Health/NIH.

Chemokines as Neuromodulators. *LG Kirby*¹, S Heinisch¹; ¹Center for Substance Abuse Research, Temple University School of Medicine, Philadelphia, PA 19140.

Chemokines are a class of small, secreted cytokines mediating leukocyte mobilization to sites of inflammation in the periphery. Chemokines and their receptors are also present in the central nervous system, localized to neurons and glia. Some chemokines constitutively colocalize with neurotransmitters and neuropeptides including acetylcholine, dopamine and vasopressin. These findings have been expanded in our laboratory to indicate expression of chemokines and their receptors in serotonin neurons

as well as colocalization of chemokine and opioid receptors on individual neurons in several brain areas. Several laboratories have demonstrated that chemokines modulate neurotransmission through effects on neuronal membrane properties, GABA/glutamate synaptic activity and Ca⁺⁺ currents in a variety of brain regions. To extend our anatomical findings, we showed that chemokines regulate serotonin neurons via actions on GABAergic synaptic activity. In support of earlier molecular and behavioral studies, we further showed desensitization of opioid receptor function by chemokines at the single-cell level in brain slices. These anatomical and functional studies have led to the hypothesis that the endogenous chemokine system represents a "third major system" of communication in the brain, acting in concert with neurotransmitter and neuropeptide systems to govern brain function. Chemokine over-expression in a number of chronic immune diseases may have additional pathological consequences through dysregulation of the neurotransmitter and neuropeptide systems to which they have been linked. Supported by NIH DA 20126, 06650, 13429 and Pennsylvania Department of Health Research Formula Fund.

Stage Specific Effects of HIV and Opiates on the Differentiation and Function of CNS Progenitors.

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Injection drug abuse and HIV infection are interlinked epidemics. HIV patients who abuse opiates appear to have higher incidence of neurologic dysfunction. HIV infection is almost exclusively propagated in immune cells. CNS pathology is thus largely secondary to processes driven by glial cells or to toxic effects of released viral proteins. The potential for synergy between HIV and opiates in the CNS is extensive since opioid receptors are widely expressed by neural and glial precursors, their mature derivatives, and microglia. CNS neural/glial progenitors are not thought of as classical HIV targets, but our studies show that critical progenitor behaviors are affected by exposure to HIV proteins, and that opiate co-exposure is synergistic for certain outcomes. In vitro, HIV-1 Tat, but not morphine or gp120, stimulates progenitors to secrete CCL5, MIP-1 α and MIP-1 β , with effects on CCR5-mediated microglial migration. HIV-1 Tat and morphine separately reduce progenitor motility, but have a robust interactive effect on proliferation and lineage progression among Sox2⁺ and Olig2⁺ progenitors. In vivo studies using inducible Tat transgenic mice have confirmed certain findings. Tat expression reduces overall and Olig⁺ proliferation (Ki67⁺) in striatum; Tat and morphine interaction reduces oligodendrocyte numbers in corpus callosum. Overall, many aspects of CNS progenitor function are vulnerable to individual or combined effects of Tat and opiates. Depending on timing, exposure may alter neuron/glial populations in specific regions, affecting gliosis, inflammation and CNS repair. Supported by NIH DA24461.

The Effect Of Δ -9-Tetrahydrocannabinol (THC) on SIVmac251 Infection in Chinese Macaques. *L Liu*¹, Q Wei², Z Cong², P Molina³, C Qin², ZW Chen¹; ¹AIDS Institute of Li Ka Shing Faculty of Medicine, The University of Hong Kong, Hong Kong, China, ²Institute of Laboratory Animal Science, CAMS and Peking Union Medical College, Beijing, China 100021, ³Department of Physiology, Louisiana State University Health Sciences Center, New Orleans, LA 70112-1393.

Δ -9-tetrahydrocannabinol (THC) is the main psychologically active ingredient of the cannabis plant. THC has been used as the active ingredient of Marinol as an appetite stimulant for AIDS patients yet its impact on HIV infection remains unclear. Here, we aim to determine the chronic effect of THC on SIVmac251 infection in Chinese macaques (0.32mg/kg im, 2 \times daily, 428 days). Sixteen animals were divided into four study groups: THC+SIV⁺, THC+SIV⁻, THC-SIV⁺ and THC-SIV⁻ with four macaques in each group. One month post THC administrations, macaques in group one and three were challenged with SIVmac251/CNS derived from the brain of an end-staged SAIDS Chinese macaque with neurological disorders. On average, group one macaques maintained better body weight when compared with group three animals. There were no significant differences of body mass index, body fat distribution, peak and steady state plasma viral loads and proviral loads between THC treated and untreated infected animals. All infected macaques, however, displayed significant drop of CD4/CD8 T cell ratio, loss of central memory CD4⁺ T cells and higher levels of Ki67⁺ CD8 T cells overtime when compared to uninfected macaques. Moreover, THC administration likely attenuates the production of peripheral IgE. Three infected macaques, 1/4 in group one with a paralyzed limb and 2/4 in group three, died of SAIDS with high viral loads before the cessation of THC. The withdrawal of THC has led to reduced food intake among THC treated animals, which requires further follow-up studies.

Our Search for the "Ideal Analgesic" in Pain Treatment. *HH Loh*¹; ¹Department of Pharmacology, University of Minnesota, Minneapolis, MN 55455.

Opioid analgesics are efficacious in controlling pain, but have adverse effects, eg, addiction. Much research has focused on designing an opioid analgesic with morphine's efficacy, but without morphine's adverse effects - an ideal analgesic. The analgesic action of opioid agonists (eg, morphine) is mediated via the mu-opioid receptor (MOR). Opioid antagonists (eg, naloxone) block the agonist effect via its interaction at MOR, but have no efficacy themselves. We found that a mutation of a conserved serine in the (TM04) region of all opioid receptors confers agonistic properties to antagonists. The observed agonists properties was due to the mutations of the conserved serine to leucine (or ala) in the 4th TM domain (S196L). We introduced MOR-S196A mutants into mouse MOR gene. In homozygous mice the opioid antagonists elicited antinociceptive effects like agonists. Importantly, chronic treatment of these mice with antagonists killed pain but did not produce the tolerance and dependence associated with morphine treatment. We have had success using gene therapy approaches and can see expression of this mutant receptor in the nociceptive neurons of the spinal cord. By administering opioid antagonists, which are devoid of opioid side effects, activation of this mutant MOR occurred while the endogenous wild type MOR were not activated, providing a proof of principal that this should be a feasible approach making an ideal pain killing paradigm for treatment of pain. Our group has developed a new target to screen for new non-addictive narcotic analgesic, which I'll present in my talk. Supported by NIH/NIDA.

Genetic Validation in the Subtypes of Alcoholism. *RB Lu*¹; ¹Department of Psychiatry, College of Medicine, National Cheng-Kung University, Tainan, Taiwan, 70428.

Alcoholism is a high heterogeneity and high genetic component mental illness. No subtype has genetic validation. We reclassified the subtype of alcoholism divided into Pure alcoholism, Anxiety-depression alcoholism, antisocial alcoholism and set up genetic validation. We found pure alcoholism is related to the MAOA gene; the ADH1B and ALDH2 genes are associated with Anxiety-depression and pure alcoholism but antisocial alcoholism is only weakly associated with the ALDH2 gene. Anxiety-depression alcoholism is related to the DRD2 A1/A1 genotype and after stratifying by the ALDH2 *1/*1 genotype which is the same association with antisocial alcoholism. One of the major differences between the two subtypes of alcoholism is that antisocial alcoholism was linked to the 3-repeat of the MAOA-uVNTR gene while the anxiety-depression alcoholism was associated with the interaction of the MAOA and ALDH2 genes. These results may be interpreted as the reason that about 54% of antisocial personality disorder comorbid with anxiety disorder and the lifetime prevalence rate of antisocial personality disorder in Han Chinese is only 0.100 but 3.3% in Western population because about only 50% Han Chinese population have the ALDH2 *1/*1 genotype but almost 99%-100% of the Western population belong to the ALDH2 *1/*1 genotype. Rice et al. (2001) reported that the lower prevalence of a disease shows the higher statistical meaning for a fixed heritability and a fixed number of trait loci. Han Chinese may provide a specific contribution to the worldwide study of alcoholism at the genetic molecular level. Supported by National Cheng Kung University Project for Promoting Academic Excellence and Developing World Class Research Centers, Taiwan.

Dynamics of Dendritic Cells and T Cells In HTLV-1-Associated Neuroinflammatory Disease: Implications in Immunomodulatory Therapies and Diagnostic Tools. *SL Manuel*¹, G Makedonas², MR Betts², J Gardner², JJ Goedert³, ZK Khan¹, B Wigdahl⁴, P Jain¹; ¹Drexel Institute for Biotechnology & Virology Research, Drexel University College of Medicine, Doylestown, PA 18902, ²Department of Microbiology and Immunology, University of Pennsylvania School of Medicine, Philadelphia, PA 19104, ³Division of Cancer Epidemiology and Genetics, National Cancer Institute, Bethesda, MD 20892, ⁴Institute for Molecular Medicine & Infectious Disease, Drexel University College of Medicine, Philadelphia, PA 19102.

HTLV-1-associated myelopathy/tropical spastic paraparesis (HAM/TSP), is a debilitating neurodegenerative disease characterized by a robust immune response including the oligoclonal expansion of cytotoxic T lymphocytes (CTLs) specific for the viral oncoprotein Tax. However, the underlying mechanism resulting in the disease process is currently unknown. The CTL response is affected by many factors including the efficiency of epitope processing and presentation. In this respect, dendritic cells (DCs), the most potent antigen presenting cells, have long been recognized as key regulators of the immune system. We have previously demonstrated that DCs are capable of priming a

pronounced Tax-specific CTL response in naïve PBLs and in HLA-A*0201 transgenic mice. Since DCs are such crucial cells of the immune system, an extensive assessment of their function and interaction with T cells in HAM/TSP is critical. Therefore, utilizing a newly standardized DC and a pre-standardized T cell polychromatic antibody cocktail, we have investigated the immune activation of these cells in HTLV-1 infected samples from the Jamaican region including seronegative controls, asymptomatic carriers (ACs), and HAM/TSP patients. The extensive immune cell profiling was compared to the matched proviral loads and Tax mRNA levels leading to the identification of unique signatures distinguishing ACs from HAM/TSP patients. Collectively, these studies possess great potential to enable immune cell monitoring and development of diagnostic and therapeutic strategies for the HTLV-associated neuroinflammatory disease. Supported by NIH grant R01 AI077414 (NIAID) to Pooja Jain and a Philadelphia NeuroAIDS Research Training Grant T32 MH079785 (NIMH).

Neuroaids and Drug Abuse: Risk Factors for Neurocognitive Impairment. *EM Martin*¹, V Meyer-Grauzas¹, PM Maki¹; ¹Department of Psychiatry, University of Illinois, Chicago, IL 60612.

Essentially all substances of abuse in combination with HIV result in significant cellular damage that can exceed effects of one or the other via multiple mechanisms including increased immunosuppression, enhanced viral replication, release of various neurotoxic cytokines and breakdown of blood-brain barrier. However, findings from the literature on brain function and neurocognition among HIV-infected substance dependent individuals (SDIs) are considerably more variable. This presentation will address current knowledge of risk factors influencing vulnerability to HIV-associated neurocognitive dysfunction among SDIs, with a primary focus on gender. Findings will be presented from recent studies in Chicago of potential gender differences in neurocognitive probes of neostriatal integrity among HIV+ SDIs and differential susceptibility of HIV+ women to effects of cocaine dependence on neurocognitive dysfunction. Supported by National Institute on Drug Abuse.

Drug Abuse Modulates Maternal Immunity and its Influence on Neonatal Immune Development. *T Molitor*¹; ¹Department of Medical Microbiology and Immunology, University of Minnesota College of Veterinary Medicine, St. Paul, MN 55108. [abstract to be distributed during conference]

Investigational Agents for Chronic Viral Infections: HIV and HCV. *SN Naggie*¹; ¹Duke Clinical Research Institute, Duke University Medical Center, Durham, NC 27710.

Chronic infections from hepatitis C virus (HCV) and human immunodeficiency virus (HIV) are a global health problem. The introduction of protease inhibitors to HIV treatment combinations in 1996 significantly reduced morbidity and mortality due to HIV infection, meanwhile the current therapy for HCV is effective in less than 50% of genotype 1 infected patients. Over the past decade advances in HIV therapeutics have produced new drug classes and fixed dose combinations, changing the face of HIV medicine. The next decade in HCV therapeutics promises to be quite similar. With advances in cell culture systems over the past decade, the development of directly acting antiviral (DAA) agents has become possible. There are currently over 50 active clinical trials in this therapeutic area, and NS3/4A protease inhibitors will be the first DAA FDA approved in 2011. This will summarize (1) the most current information regarding the effectiveness of protease inhibitors in treating chronic HCV, (2) the possibility of combination therapies for HCV, and (3) the now smaller pipeline for HIV therapies and the role these therapies may play in the management of HIV-infected patients.

Human Immunodeficiency Virus Type 1 (HIV-1) Infects Human Brain Pericytes In Vitro. *S Nakagawa*¹, M Toborek¹; ¹Department of Neurosurgery, University of Kentucky, Lexington, KY 40536.

Human immunodeficiency virus type 1 (HIV-1) infection of the central nervous system (CNS) causes disruption of the blood-brain barrier (BBB) and contributes to the development of neurological dysfunctions. The BBB strictly regulates the transport of blood-borne cells and substances into the brain. Brain capillary endothelial cells are a major component of the BBB and have a dynamic interaction with other neighboring cells, namely, astroglia, pericytes, perivascular microglia, and neurons. The cross-talk between cells of the neurovascular unit is crucial for the formation and maintenance of a functional BBB. There are many reports related to the effects of HIV-1 on astrocytes, microglia, neurons, and endothelial cells. However, no study addressed the effects of HIV-1 infection on pericytes. The purpose of this study was to evaluate whether pericytes can be infected with HIV-1. To determine the expression of HIV-1 receptors on human brain pericytes, confocal microscopy and western blots were performed. Human

brain pericytes expressed the HIV-1 receptor and co-receptors (CD4, CXCR4, and CCR5). Confirmation of HIV replication in human brain pericytes was performed by HIV-1 p24 ELISA. Low viral replication was detected after exposure to the X4 and R5 strains of HIV. These findings indicate that human brain pericytes can be infected with HIV-1. Infected pericytes may be involved in progression of the HIV-1-induced CNS damage. Supported by in part by ES 07380, DA027569, MH63022, MH072567, and NS39254.

Fulminant Encephalopathy in HIV-Infected Cocaine Abusers. *A Nath*¹, *S Newsome*¹, *C Pardo*¹, *JC McArthur*¹; ¹Department of Neurology, Johns Hopkins University, Baltimore, MD 21287.

Background: The clinical spectrum of neurological complications of active drug abusers in HIV-infected patients is not well defined. Further, multiple comorbidities may contribute to these clinical subtypes. Methods: In a retrospective review conducted at Johns Hopkins Hospital from 1993 to 2008 HIV-infected patients who were actively abusing drugs and had bilateral basal ganglia lesions on MRI were identified. The following databases were searched: radiology, autopsy and the Moore HIV clinic database. Clinical, laboratory and radiographic findings were correlated to define the syndrome. Results: Ten individuals were identified who presented with a change in mental status or seizures, used cocaine or cocaine with heroin, had uncontrolled HIV infection (>190,000 copies/mL of plasma), elevated CSF protein (63-313 mg/dL), and diffuse hyperintense bilateral basal ganglia lesions on imaging. The majority of patients (8/10) had renal failure and despite supportive therapy most (7/9) ultimately died (median survival 21 days). Postmortem examination in one individual showed the presence of overwhelming microglial activation in the basal ganglia. The two surviving individuals were started on combined antiretroviral therapy (CART) during hospitalization. Conclusion: We describe a rare but unique clinical syndrome of a fulminant encephalopathy associated with prominent basal ganglia involvement in HIV-infected drug abusers. This syndrome is associated with high mortality. Early CART institution may be useful and neuroprotective in this disorder, although this requires further investigation. Supported by NIDA, NINDS.

Alcohol, Immunosuppression and HIV. *S Nelson*¹; ¹Comprehensive Alcohol Research Center, Louisiana State University Health Sciences Center, New Orleans, LA 70112.

Alcohol abuse and HIV-infection are major public health problems and frequently coexist in the same individual. While several studies have shown a significant association between alcohol consumption and the risk of being infected with HIV, it is unclear whether this association is due to behavioral and/or biomedical mechanisms. Studies of HIV-infected patients are limited in their ability to control for timing and dose of HIV exposure, nutrition, concurrent use of other drugs of abuse, use of antiretroviral therapy, as well as the frequency and amount of alcohol consumed. In order to study the impact of alcohol on HIV infection, we developed a model of chronic alcohol consumption in rhesus macaques infected with simian immunodeficiency virus (SIV). Our studies have shown that plasma viral loads are significantly higher in alcohol-consuming macaques 60-120 days post SIV infection (viral set point) compared to control animals. The viral set point is predictive of disease progression and in our studies alcohol consumption was associated with accelerated disease progression to end-stage disease. Alcohol consumption and SIV/HIV have been shown to compromise intestinal barrier function resulting in increased transfer of endotoxin and other antigens into the systemic circulation which triggers a cascade of inflammatory responses, which is a main driving force for HIV replication and disease progression. Therapeutic approaches focused at restoring the integrity of the mucosal barrier might prove effective in mitigating HIV replication and disease progression. Supported by NIH/NIAAA/P60 AA009803.

Sympathetic Nervous System and Norepinephrine: Adrenergic Receptor-Mediated Effects on Immune Function. *VM Sanders*¹; ¹Department of Molecular Virology, Immunology and Molecular Genetics, Ohio State University Medical Center, Columbus, OH 43210.

Four key discoveries indicate that a mechanism exists by which the sympathetic nervous system is able to communicate with cells of the peripheral immune system: 1) The parenchyma of primary and secondary lymphoid organs are innervated with sympathetic nerve fibers; 2) The sympathetic neurotransmitter norepinephrine is released from nerve terminals residing within lymphoid tissue upon antigen or cytokine administration; 3) Lymphoid cells, except the Th2 cell, express the beta-2-adrenergic receptor (β 2AR) that binds norepinephrine; and 4) Norepinephrine regulates lymphocyte activity and the magnitude of an immune response. Recent findings on β 2AR adrenergic receptor expression on

lymphocytes and unexpected mechanisms by which β 2AR engagement regulates the level of immune cell activity will be discussed. Supported by NIH AI37326.

Neuron-Glia Crosstalk in HIV-1 Neuropathogenesis. *P Seth*¹, *P Garg*¹; ¹NeuroAIDS Laboratory, Cellular and Molecular Neuroscience, National Brain Research Centre, Manesar, India 122050.

Cell culture model system has been of immense importance in understanding neuro-pathogenesis underlying HIV Associated Neurocognitive Disorder (HAND). Previous studies have reported an increase in Cx 43 expression at protein level upon exposure of primary human astrocytes with live HIV virus. However, the underlying signaling pathways and the consequences of the augmented gap junction communication still remain unexplored. To gain better insights into the molecular mechanisms underlying HIV mediated neurotoxicity, we used human neuron-astrocyte co-culture model system using cells differentiated from human fetal brain derived neural precursor cells, thereby closely mimicking the in vivo conditions of human brain. Gap Junction Channels (composed of connexins as their key proteins) are known to be one of the major mode of intercellular communication as they allow the passage of various ions and second messengers, thereby regulating signaling pathways. In our study we have observed a significant increase in gap junction communication (Cx 40, 26, 36 and 43) post treatment with HIV-1 Tat protein. This increase in gap junction communication promotes cell death as elucidated by the TUNEL assay. Further, cell proliferation was also inhibited because of increased connexin expression as evidenced by Ki67 staining. This effect is confirmed by the use of α -glycyrrhetic acid (Gap Junction blocker) that restores cell viability and proliferation in the treated cells. Studies on signaling pathways and role of cell cycle regulatory proteins are currently in progress in our lab. Supported by Research grants from Department of Biotechnology and National Brain Research Centre, India.

HIV-1 Glycoprotein 120 Induces the Pro-Inflammatory Cytokine IL-6 via the NF- κ B Pathway and Methamphetamine can Synergistically Potentiate gp120-Mediated IL-6 Induction. *A Shah*¹, *A Kumar*¹; ¹Division of Pharmacology & Toxicology, University of Missouri - Kansas City, Kansas City, MO 64108.

HIV-1 gp120 facilitates the viral attachment and entry into the CNS leading to infection of various regions of the brain, resulting in HIV-1 Associated Neurocognitive Disorders (HAND). In this study, we sought to address whether gp120 can induce the pro-inflammatory cytokine interleukin-6 (IL-6). We also addressed whether exposure to methamphetamine can induce IL-6 expression in astrocytes, either alone or in synergy with gp120. The transfection of gp120 showed a time dependent induction of IL-6 mRNA and protein expression, with peak levels of 51.3 ± 2.1 fold and 11.6 ± 2.2 fold respectively. The induction of IL-6 could be successfully abrogated by gp120-specific siRNA. Using chemical antagonists and siRNA for the NF- κ B pathway, we demonstrated the involvement of this transcription factor in gp120-mediated IL-6 induction. Both I- κ B- α and IKK2 inhibitors could abrogate the gp120-mediated IL-6 induction by 56.5% and 60.8% respectively. In this study, we also report that exposure of SVGA cells to methamphetamine results in an increase in IL-6 expression by 4.9 ± 2.3 fold, which was blocked by mGluR5 inhibitor MPEP. The expression of IL-6 increased further (76.0 ± 12.1 fold) when meth exposure was combined with gp120 transfection. Together our results suggest NF- κ B to be a potential therapeutic target for HIV-1 infected patients. We also demonstrate that methamphetamine can act synergistically with gp120 to induce IL-6 expression. Thus, HIV-infected individuals who consume methamphetamine are at higher risk of developing neuro-inflammation than non-abusers.

Molecular Chaperone and Interorganelle Signaling in Disease. *T Su*¹; ¹Cellular Pathobiology Section, Intramural Research Program, National Institute on Drug Abuse, National Institutes of Health, Baltimore, MD 21224.

The inter-organelle signaling plays important roles in many physiological functions. The endoplasmic reticulum (ER)-mitochondrion signaling affects intramitochondrial Ca²⁺ homeostasis and cellular bioenergetics. The ER-nucleus signaling attenuates ER stress. The ER-plasma membrane signaling regulates cytosolic Ca²⁺ homeostasis and the ER-mitochondrion-plasma membrane signaling the hippocampal dendritic spine formation. The sigma-1 receptor (Sig-1R), an ER chaperone protein, act as an inter-organelle signaling modulator. Sig-1Rs normally reside at the ER-mitochondrion contact called the MAM (mitochondrion-associated ER membrane) where Sig-1Rs regulate ER-mitochondrion signaling and the ER-nucleus cross-talk. When cells are stimulated by Sig-1R agonists or under prolonged stress, Sig-1Rs translocate from the MAM to the ER reticular network and plasma membrane to regulate a

variety of functional proteins including ion channels, receptors, and kinases. Cocaine is a Sig-1R agonist. Cocaine causes the translocation of a portion of Sig-1Rs from the MAM to other areas of neurons including the general ER membrane, plasma membrane, nucleus, and mitochondria. Thus, in addition to the well known action of cocaine in increasing BDNF and DA in the brain, cocaine may achieve its addictive action in part by interacting with ion channels or receptors from inside of the neuron via its interaction with Sig-1Rs. Supported by Intramural Research Program, NIDA, NIH, DHHS.

Natural History and Pathogenesis of HIV and HCV. *DL Thomas*¹; ¹Infectious Diseases, Johns Hopkins School of Medicine, Baltimore, MD 21205.

Hepatitis C virus (HCV) coinfection occurs in an estimated one quarter of HIV-infected persons in Europe, Australia, and the United States. HCV is especially a problem for injection drug users as nearly all HIV infected drug users in some settings already has HCV infection. As use of highly active antiretroviral drugs has markedly reduced opportunistic infections, HCV-related liver disease has emerged as a leading cause of death. HIV infection adversely affects both the natural history and the treatment of hepatitis C. Because there are no experimental models of coinfection and because the pathogenesis of each infection is incompletely understood, how HIV infection alters hepatitis C is not clear. Possible mechanisms will be discussed. Supported by NIDA.

The Cholinergic Anti-Inflammatory Pathway and Innate Immunity. *KJ Tracey*¹; ¹Center for Biomedical Sciences, Feinstein Institute for Medical Research, Manhasset, NY 11030. [abstract to be distributed during conference]

Neuropsychiatric Complications Of HIV, HCV, and Antiviral Treatment. *G Treisman*¹; ¹Department of Psychiatry and Behavioral Sciences, Johns Hopkins University School of Medicine, Baltimore, MD 21287.

Infectious diseases transmitted by specific behaviors such as Hepatitis C and HIV have been shown to be associated with psychiatric conditions. Psychiatric disorders including addiction, major depression, personality disorder, cognitive impairment, dementia, and major mental illness increase risk behaviors for infection. Additionally, CNS inflammation, depletion of resources, hopelessness, and therapeutic nihilism increase these psychiatric conditions, leading to poor adherence to treatment and treatment failure. Finally, the medications used to treat Hepatitis C and HIV cause psychiatric and neurologic side effects that further complicate care. Treatment is made more complex by conditions such as neuropathic pain produced by antiviral medications, depression caused by interferon, and delirium caused by ancillary medications. Additionally, the polypharmacy required for treatment of substance abuse disorders, affective disorders, schizophrenia, Hepatitis C and HIV create a variety of complex drug interactions that undermine treatment. An integrated treatment plan with an emphasis on collaborative efforts at surveillance for side effects, drug interactions, treatment of non-adherence, and the emergence of treatment related complications has been shown in model programs to improve outcomes and effectiveness of treatment. Coherent treatment of psychiatric, neurologic, and substance abuse disorders may also decrease the risk of transmission and the development of viral resistance.

Molecular Neurobiology of Drug Addiction: Role of NMDA Receptors. *JQ Wang*¹, *LM Mao*¹; ¹Department of Basic Medical Science, University of Missouri-Kansas City School of Medicine, Kansas City, MO 64108.

Plastic changes in glutamate receptors are critical for the remodeling of excitatory synapses in limbic reward circuits in response to chronic psychostimulant exposure. Such remodeling is thought to directly link to enduring drug-seeking behavior, although underlying mechanisms are poorly understood. In this study, we investigated an ionotropic glutamate receptor subtype, N-methyl-D-aspartate receptors (NMDAR), in their adaptations to chronic psychostimulant exposure and their roles in mediating drug-stimulated synaptic and behavioral plasticity. By focusing on the turnover and trafficking of NMDARs, we found that chronic exposure to the psychostimulant amphetamine (4 mg/kg, i.p., once daily for 7 days; 14 days of withdrawal) induces selective downregulation of NMDAR NR2B subunit proteins in the confined surface membrane pool of rat striatal neurons at synaptic sites. Remarkably, this downregulation is a long-lived event and results from the destabilization of surface-expressed NR2B due to accelerated ubiquitination and degradation of crucial NR2B-anchoring proteins by the ubiquitin-proteasome system. The biochemical loss of synaptic NR2B further translates to the significant modulation of synaptic plasticity in the form of long-term depression at cortico-accumbal glutamatergic synapses. Behaviorally,

genetic disruption of NR2B induces, whereas restoration of NR2B loss prevents, behavioral sensitization to amphetamine. Our data identify NR2B as a key regulator in the remodeling of excitatory synapses and persistent psychomotor plasticity in response to amphetamine. Supported by NIH/R01 DA010355 and R01 MH061469.

Challenges in the Diagnosis of HAND in a Hispanic Cohort of HIV-Seropositive Women. *V Wojna*¹, *R Mayo*¹, *RL Skolasky*², *N Haughey*², *A Nath*²; ¹NeuroAIDS Program and Neurology Division, University of Puerto Rico Medical Sciences Campus, San Juan, PR 00936-5067, ²Neurology Department, Johns Hopkins University, Baltimore, MD 21287.

HIV-associated Neurocognitive Disorders (HAND) continues to be a serious complication of HIV infection. Infected patients using combined antiretroviral treatment (CART) are at risk of developing milder forms of HAND since they are living longer and may present other risk factors for cognitive impairment such as aging effects and drug abuse. The recommended nosology for the diagnosis of HAND weights heavily on the performance of neuropsychological (NP) tests. This may represent a challenge in settings where there is limited NP normative data. We have designed a cohort of HIV-seropositive women to study HAND since women represent the fastest growing groups with HIV/AIDS. Using the experience of the Hispanic-Latino Longitudinal Cohort of HIV-seropositive Hispanic Women we will discuss how we establish the NP battery tests and the diagnosis of HAND, how we deal with the normative issue, and how HAND criteria varies from previously establish criteria. While our cohort does not recruit participants with active drug abuse, a considerable amount have tested positive to illicit drugs. Discussions will also include the effects of drugs of abuse on HAND diagnosis and CSF antioxidants and oxidative stress markers. Although, we are studying a cohort of Hispanic women we understand that our findings are relevant to HIV-seropositive women in general. Supported by NIH grants U54NS43011, P20RR11126, S11NS046278.

Cocaine-Mediated Induction of Platelet-Derived Growth Factor: Implication for Increased Vascular Permeability. *H Yao*¹, *M Duan*¹, *SJ Buch*¹; ¹Pharmacology, University of Nebraska Medical Center, Omaha, NE 68198.

Neuroinflammation associated with advanced HIV-1 infection is often exacerbated in cocaine-abusing HIV-infected individuals. The underlying mechanisms could, in part, be attributed to the increased impairment of blood brain barrier integrity in the presence of cocaine. Platelet-Derived Growth Factor (PDGF) has been implicated in a number of pathological conditions, specifically attributable to its potent mitogenic effects. Its modulation by drug abuse, however, has received very little attention. In the present study, we demonstrated cocaine-mediated induction of PDGF-BB in human brain microvascular endothelial cells through the binding to its cognate sigma receptor. Furthermore, this effect was mediated, with subsequent activation of mitogen-activated protein kinases (MAPKs) and Egr-1 pathways, culminating ultimately into increased expression of PDGF-BB. Cocaine exposure resulted in increased permeability of the endothelial barrier and this effect was abrogated in mice exposed to PDGF-BB neutralizing antibody, thus underscoring its role as a vascular permeant. In vivo relevance of these findings was further corroborated in cocaine-treated mice that were administered neutralizing antibody specific for PDGF-BB as well as in Egr-1 ^{-/-} mice. Understanding the regulation of PDGF-BB expression may provide insights into the development of potential therapeutic targets for neuroinflammation associated with HIV infection and drug abuse. Supported by NIDA/DA020392, DA023397, DA024442, and DA030285.

The Effects of Excessive Alcohol Consumption on the Development of Myelosuppression During SIV Infection. *P Zhang*¹, *RW Siggins*¹, *DA Welsh*², *GJ Bagby*¹, *JP Dufour*³, *S Nelson*²; ¹Department of Physiology, Louisiana State University Health Sciences Center, New Orleans, LA 70112; ²Department of Medicine, Louisiana State University Health Sciences Center, New Orleans, LA 70112; ³Division of Veterinary Medicine, Tulane National Primate Research Center, Covington, LA 70433.

Myelosuppression is common in patients with human immunodeficiency virus (HIV) infection. Granulocytopenia has been reported to occur in 60-80% of HIV-infected individuals. Alcohol abuse is a co-factor for progression of acquired immunodeficiency syndrome (AIDS). Alcohol injures the bone marrow and impairs myelopoiesis. This study investigated the effect of chronic alcohol consumption on the development of myelosuppression in rhesus macaques infected with simian immunodeficiency virus (SIV). SIV infection caused a significant decrease in blood granulocyte counts, which occurred in

association with the loss of CD4+ cells. Alcohol consumption caused a decrease in marrow hematopoietic stem cells. Alcohol exaggerated the SIV-induced reduction of the granulocyte storage pool in the bone marrow and impaired the granulopoietic response to secondary bacterial infection in the lung. Alcohol consumption significantly increased CpG methylation in the promoter region of the C/EBP α (a master transcript factor for myeloid lineage development) gene and suppressed C/EBP α gene expression by bone marrow cells. In addition, alcohol disturbed the hematopoietic microenvironment by promoting a myeloid differentiation block. These data show that alcohol exaggerates the development of myelosuppression during SIV infection. Compounding the direct lymphotoxicity of SIV, a defective granulocytic response is an additional risk factor for acceleration of AIDS progression. Supported by NIH grants AA09803, AA019676, and AA07577.

Neurogenesis, Brain Inflammation and its Links to the Pathogenesis and Potential Therapy of Neurodegenerative Disorders. *J Zheng*^{1,2}, C Tian¹, Y Wang¹, L Sun¹, K Ma¹; ¹Laboratory of Neuroimmunology and Regenerative Therapy, Department of Pharmacology and Experimental Neuroscience, University of Nebraska Medical Center, Omaha, NE 68198-5930, ²Department of Pathology and Microbiology, University of Nebraska Medical Center, Omaha, NE 68198-5930.

Neurogenesis is a highly regulated process responsible for the generation of new neurons, astrocytes and oligodendrocytes from neural stem cells. It is widely accepted that neurogenesis persists into adulthood and may play a crucial role in complicated behaviors such as learning and memory. Accumulating evidence suggests that impaired neurogenesis also contributes to the pathogenesis of several brain disorders. This has been investigated with an emphasis on how brain inflammation may influence the pathogenesis of neurodegenerative diseases such as HIV-1 associated neurocognitive disorders. Direct reprogramming of a variety of somatic cells with the transcription factors Oct4 (also called Pou5f1), and Sox2 with either Klf4 and Myc or Lin28 and Nanog generates induced pluripotent stem cells (iPSCs) with marker similarity to embryonic stem cells. However, the difference between iPSCs derived from different origins is unclear. In this study, we hypothesize that reprogrammed cells retain a “memory” of their origin and possess an enhanced potential for related tissue differentiation. We reprogrammed primary mouse astrocytes via ectopic retroviral expression (OCT3/4, Sox2, Klf4, Myc) and found that the resulting mAsiPSCs expressed stem cell markers and formed teratomas in SCID mice with derivatives of all three germ layers in a manner similar to mouse embryonic stem cells. To test our hypothesis, we compared EBs formation and neuronal differentiation between iPSCs generated from mouse embryonic fibroblasts (MEFsiPSCs) and our mAsiPSCs. mAsiPSCs grew slower and possessed more potential for neuronal differentiation compared to MEFsiPSCs. Our results suggest that mAsiPSCs retain a “memory” of the central nervous system, conferring added potential for neuronal differentiation. iPSC present exciting avenues to study human disease as well as an approach for cell therapy.

POSTER SESSION I: YOUNG INVESTIGATOR ABSTRACTS

(in alphabetical order by presenting author)

Beneficial Effect of the Cannabinoid Receptor-2-Selective Agonist in Spinal Cord Injury via Immune Modulation. *S Adhikary*¹, H Li¹, M Skarica¹, RF Tuma¹, D Ganea¹; ¹Department of Physiology, Temple University School of Medicine, Philadelphia, PA 19140.

Previous studies from our laboratory showed that treatment with the CB2R specific agonist O-1966 improved recovery of motor and bladder function in mouse models of acute spinal cord injury. In SCI, increased infiltration and activation of inflammatory immune cells that follow the initial injury exacerbate neuronal damage. In this study, a murine thoracic SCI contusion model was used and spinal cords were analyzed for expression of various cytokines and chemokines as well as infiltration of peripheral immune cells at various time-points. There were no differences in the expression of inflammatory molecules or in the number of infiltrating peripheral immune cells between controls and mice treated with O-1966 at an early time-point. However, at later time-points, we observed significant differences in the expression of CXCL family chemokine members CXCL9, 10, and 11, the CC family chemokine receptor members CCR1, 2, 3, the cytokine IL-23 and its receptor, and the Toll like receptors TLR1, 4, 6, and 7 in spinal cords from mice treated with O-1966. This decrease correlated with the reduction in number of infiltrating

myeloid cells. We also observed reduced microglia activation as determined by immunohistochemical staining for Iba1. Based on these results, we postulate that the beneficial effect of CB2R agonists in acute SCI is mediated, at least partially, through the inhibition of CNS resident microglia/astrocyte activation, leading to reduced levels of chemokines and pro-inflammatory cytokines, and reduced infiltration of peripheral immune cells. Supported by NIH/NIAID R01AI05230, NIDA T32 DA07237.

Differential Expression of Cannabinoid Genes in Alcoholics. *M Agudelo*¹, *C Spadola*¹, *A Yndart*¹, *N Gandhi*¹, *Z Saiyed*¹, *VB Pichili*¹, *T Samikkannu*¹, *MP Nair*¹; ¹Department of Immunology, College of Medicine, Florida International University, Miami, FL 33199.

Cannabinoid genes are known to be involved in alcohol use disorders; and CB1 antagonists have been shown to reduce alcohol drinking. Previous studies have focused on cannabinoids and alcohol-related effects in the brain; however, the role cannabinoid genes play on alcohol effects in the immune system is not elucidated yet. Psychoactive substances such as alcohol and cannabinoids can affect immune responses and have detrimental effects on immune cells such as dendritic cells (DCs). We hypothesize that alcohol can exert its effects on DCs by modulating changes in cannabinoid genes. Therefore, we studied the expression of cannabinoid receptors 1, 2 (CNR1 and CNR2), and the novel cannabinoid G protein-coupled receptor 55 (GPR55) in human monocyte-derived dendritic cells (MDDCs) from non-alcoholic and alcoholic blood donors. CNR1, CNR2, and GPR55 gene expression was measured by quantitative real-time PCR. Our results show a significant upregulation of CNR2 and GPR55; and a downregulation of CNR1 in MDDCs from alcoholics compared to MDDCs from non-alcoholics. These findings were further confirmed in vitro using MDDCs treated with alcohol. Results of these analyses provide insights into alcohol mechanisms of DC regulation and suggest that alcohol is inducing CNR2 and GPR55 genes in DCs. Supported by the National Institute on Drug Abuse (NIDA).

HIV-1 Induced Amyloid Beta Accumulation in Brain Endothelial Cells: Signaling Mechanisms Involved. *IE Andras*¹, *SY Eum*¹, *Y Zhong*¹, *W Huang*¹, *B Hennig*², *M Toborek*¹; ¹Neurosurgery, University of Kentucky, Lexington, KY 40536, ²College of Agriculture, University of Kentucky, Lexington, KY 40536.

Amyloid beta deposition is increased in HIV-1 infected brains. Our previous study have shown that HIV-1 exposure increased amyloid beta accumulation at the blood brain barrier (BBB) level in a model of human brain microvascular endothelial cells (HBMEC) partly by increasing the levels of RAGE, known to regulate amyloid beta transport across the BBB. In this study we proposed to investigate further the mechanisms involved in amyloid beta accumulation in HBMEC. Silencing of caveolin-1 diminished the RAGE level increase and amyloid beta uptake in HIV-1 exposed cells as compared to control. Amyloid beta accumulation was also lipid raft dependent as beta-methyl-cyclodextrin (MCD) pretreatment almost completely abolished it in control and HIV-1 treated cells. The Ras inhibitor farnesylthiosalicylic acid (FTS) and the p38 MAPK inhibitor SB203580 blocked the amyloid beta accumulation evoked by HIV-1. In addition, HIV-1 exposure increased the levels of early endosomal antigen-1 (EEA1) which partly colocalized with amyloid beta. EEA1 levels were reduced by FTS in cells exposed to HIV-1 and amyloid beta. Our data show that amyloid beta accumulation in HBMEC is lipid raft dependent, involving the caveolae-associated Ras-MAPK signaling pathway. In addition, this pathway may alter the early endosomes which are likely to be involved in the increased amyloid beta uptake in HBMEC in the presence of HIV-1. Supported by MH63022, MH072567, and NS39254.

A Gene Therapeutic Approach For HIV-1 Associated Dementia (HAD) using Targeted Nanoparticles for TIMP-1 Delivery into CNS. *F Ashutosh*¹, *K Borgmann*¹, *L Tang*¹, *V Labhasetwar*², *A Ghorpade*¹; ¹Department of Cell Biology and Anatomy, University of North Texas Health Science Center, Fort Worth, TX 76107, ²Department of Biomedical Engineering, Cleveland Clinic Lerner College of Medicine, Cleveland, OH 44195.

Astrocyte production of tissue inhibitor of metalloproteinases-1 (TIMP-1) is important in inflammatory diseases such as HIV-1-associated dementia (HAD). Previously, we have shown acute activation of astrocytes increases TIMP-1 as a neuroprotective response. However, in chronic activation TIMP-1-mediated responses fail due to down regulation of TIMP-1. In this study, we explored the feasibility of TIMP-1 gene therapy in the CNS. A dual approach using a glial fibrillary acidic protein (GFAP)-promoter driven TIMP-1 cDNA construct and CNS targeting nanoparticles (NP) promotes astrocyte-specific TIMP-1 expression. Two plasmid vectors, pGFAP-TIMP-1 and pGFAP-LUC (luciferase) were constructed for TIMP-1 over-expression and quantification / distribution of promoter activity, respectively. Constructs

were transfected into cultured human astrocytes and various non-glia cells for evaluation of GFAP-driven expression. Transfected cells were also stimulated with HIV-relevant inflammatory stimuli. Significant increases in TIMP-1 and LUC expression were found in astrocytes. The NP encapsulated plasmids were evaluated for delivery, protein expression and cytotoxicity in vitro. The neuroprotective effects of GFAP promoter driven TIMP-1 expression in astrocytes were assayed. Therefore, present studies demonstrate NP-encapsulated pGFAP-TIMP-1 can be delivered into astrocytes, is responsive to HIV-relevant inflammatory stimuli and increases TIMP-1 levels. These studies provide proof-of-concept and establish a foundation for future gene therapeutic approaches. Supported by NIH/2R01NS048837-06.

HIV-1 and Drugs of Abuse alter Neurotrophin Levels in Human Lymphocytes. V Avdoshina¹, A Garzino-Demo², A Bachis¹, Mc Monaco³, C Liu⁴, Ma Young⁴, I Mocchetti¹; ¹Department of Neuroscience, Georgetown University Medical Center, Washington, DC 20057, ²Institute of Human Virology, University of Maryland, Baltimore, MD 21201, ³Laboratory of Molecular Medicine and Neuroscience, NINDS/NIH, Bethesda, MD 20824, ⁴Department of Medicine, Georgetown University Medical Center, Washington, DC 20057.

The neurotrophins (NT) brain-derived neurotrophic factor (BDNF), nerve growth factor (NGF) and NT-3 are produced by immune organs and immunocompetent cells, including T cells and macrophages, and might play a role in various functions of the immune system including lymphocyte proliferation/survival. Little is known about the effect of HIV-1 on the levels of NT in the blood. In this study, we examined whether HIV-1 reduces NT levels in human blood cells by comparing serum levels of BDNF, NGF, and NT-3 in HIV-infected and HIV-uninfected subjects using the Women's Interagency HIV Study cohort of the Washington, DC area. We found that serum concentration of BDNF and NGF but not NT-3 are decreased in HIV-positive subjects. At least a third of HIV-positive subjects used for our investigation were drug users. To examine whether drug abuse and HIV infection interact to affect NTs, we analyzed the levels of NT in HIV-positive and negative subjects as a function of drug abuse. In HIV positive drug abusers the levels of BDNF and NGF were higher than those in HIV positive non drug users, suggesting that drugs of abuse might restore the levels of NT. To prove the hypothesis that HIV lowers NT levels, we exposed human T cells in culture to both strains of HIV (R5 and X4). Both strains reduced BDNF mRNA and protein expression within 24 hr. These data suggest that reduced level of BDNF may increase the risk of developing immunological and brain abnormalities among HIV-positive individuals. Supported by DA026174, NS066842, UO1-AI-35004, UO1-AI-31834, UO1-AI-34994, UO1-AI-34989, UO1-AI-34993, UO1-AI42590, UO1-HD32632, and UL1 RR024131.

Manufacture and Pre-Clinical Testing of Nanoformulated Antiretroviral Therapeutics. S Balkundi¹, A Nowacek¹, J McMillan¹, U Roy¹, A Martinez-Skinner¹, R Mosley¹, G Kanmogne¹, A Kabanov¹, T Bronich¹, H Gendelman¹; ¹Pharmacology and Experimental Neuroscience, University of Nebraska Medical Center, Omaha, NE 68128.

Although antiretroviral therapy has resulted in increased longevity and reduced morbidities for human immunodeficiency virus-1infection, limitations in compliance, pharmacokinetics and drug toxicities remain at issue. We have synthesized nanoformulated antiretroviral therapy (nanoART) with varied physical and chemical properties to improve pharmacodynamics and bioavailability using wet milling, homogenization, ultrasonication and micelles as nanocontainers. Nanoformulations were characterized by DLS. Uptake, retention, and release of nanoART into and from human monocyte-derived macrophages (MDM) were determined. Antiretroviral activities of the nanoART in MDM were determined by RT and HIV-1p24 antigen assays. Wet milled and homogenized nanoART formulations of indinavir, ritonavir, atazanavir, and efavirenz ranged in size from 252- 418 nm with PDI of 0.152- 0.29 and zeta potentials from -15.3 to -40.6 mv. The micellar formulations were <30 nm with PDI values of 0.019 to 0.08. NanoART uptake by MDM was measured over 8h and cell retention and release were measured over 15 days. Drug levels in nanoART-laden MDM were detectable by HPLC for crystalline and PLGA formulations up to 15 days but were limited in micelles. Efavirenz crystalline nanoparticles most effectively inhibited HIV-1 replication as demonstrated by reduction of RT activity and p24 expression. The repackaging of clinically available antiretroviral medications into nanoparticles for treatment of HIV-1 disease may improve compliance notably in the drug abusing population and positively affect disease outcomes.

Morphine Modulation of IL17 Expression and Signaling in Alveolar Epithelial Cells. S Banerjee¹, S Roy¹; ¹Surgery, University of Minnesota, Minneapolis, MN 55455.

Chronic Morphine has been shown to increase the pathogenic load in lung infections, while its role in modulating the barrier integrity has largely remained unexplored. The protective role of IL17A in this context has been well documented in the past two decades. The existence of IL17 in the realm of both innate and adaptive immunity ($\gamma\delta$ T Cells and $\alpha\beta$ T Cells respectively) presents unique perspective in its regulation and signaling. In the innate realm, there are several reports of stimulated or constitutive expression of IL17A by various cell types of hematopoietic/myeloid origin ($\gamma\delta$ T cells, macrophages, neutrophils), as well as epithelial cells (Paneth cells in the intestinal mucosa). Furthermore, IL17 mediated immune responses, namely IL6, IL8, GM-CSF, defensins, S110A and lipocalins, get induced within an hour of mucosal barrier compromise or activation of the Pattern Recognition Receptors. Our study specifically focuses on the barrier function of the human alveolar epithelium, whereby, we show that these cells are capable of inducing the 'first response' to extracellular pathogens, even in the absence of resident immune cells, involving IL17 and its signaling, possibly in an autocrine fashion. We also show that these cells possess the functional apparatus to induce IL17 production in response to external stimuli, including constitutive expression of the key transcription factor ROR γ t. And finally, we elucidate the effects of chronic Morphine in negatively modulating these responses. Supported by NIH grants: RO1 DA 12104 ; RO1 DA 022935; KO2 DA 015349, and P50 DA11806.

The Effect of HIV-1 Tat on Intracellular Production and Distribution of Abeta 1-42 in Hippocampal Neurons. *SJ Bertrand*¹, *MV Aksenova*¹, *MY Aksenov*¹, *CF Mactutus*¹, *RM Booze*¹; ¹Department of Psychology, University of South Carolina, Columbia, SC 29201.

Epidemiological studies indicate a growing incidence of amyloid beta peptide mediated brain pathology in aging HIV patients. Effects of viral proteins (particularly Tat) on Abeta biogenesis may be the key pathway that links chronic HIV-1 infection to the onset of cognitive deficits in aging cohort of HIV-positive patients. Our recent findings demonstrate that HIV-1 Tat, clade B, promotes premature release of Abeta1-42 and the accumulation of amyloid aggregates in long-term rat hippocampal cell cultures. Our current study analyzes the level and distribution of Abeta1-42 immunoreactivity in mature (21 DIV) hippocampal neurons that incorporate Tat1-86B following the acute exposure to a toxic (50 nM) dose of Tat1-86B. Co-labeling of control and Tat treated rat hippocampal cultures with anti-Tat and anti-Abeta1-42 antibodies has shown increased intensity of the Abeta1-42 reactivity in Tat-containing cells. Results of Abeta1-42 immunolabeling revealed the presence of Abeta-positive conglomerates associated with cell bodies and processes in 21 DIV Tat-treated cell cultures. Hippocampal cells co-labeled with anti-Abeta1-42/anti-Tat display increased Abeta1-42 reactivity in dendrites as a result of Tat exposure. Results of Congo Red/F-actin staining show beta-amyloid specific labeling of the processes proximal and distant to cell bodies in Tat treated cultures. Our cytomorphological observations support the hypothesis that neurotoxic forms of misfolded Abeta can mediate Tat-induced synaptodendritic injury. Supported by University of South Carolina Research Foundation Grant and NIH Grant # DA013137.

HIV-1 Tat Mediated Induction of Platelet-Derived Growth Factor in Astrocytes: Role of Early Growth Response Gene 1. *C Bethel-Brown*¹, *H Yao*², *S Callen*², *YH Lee*³, *PK Dash*², *A Kumar*⁴, *S Buch*²; ¹Molecular and Integrative Physiology, University of Kansas Medical Center, Kansas City, KS 66160, ²Pharmacology and Experimental Neuroscience, University of Nebraska Medical Center, Omaha, NE 68137, ³Institute of Biomedical Science and Technology, Konkuk University, Seoul, Korea 143-701, ⁴Pharmacology, University of Missouri Kansas City, Kansas City, MO 64108.

HIV-associated neurological disorders (HAND) are estimated to affect almost 60% of HIV infected individuals. HIV-encephalitis (HIVE), the pathological correlate of the most severe form of HAND is often characterized by glial activation, cytokine/chemokine dysregulation, and neuronal damage and loss. However, the severity of HIVE correlates better with glial activation rather than viral load. Using the macaque model, it has been demonstrated that simian immunodeficiency virus encephalitis (SIVE) correlates with increased expression of the mitogen platelet-derived growth factor-B (PDGF-B) chain in the brain. The present study was aimed at exploring the role of PDGF-B chain in HIV-associated activation and proliferation of astrocytes. Specifically, the data herein demonstrate that exposure of rat and human astrocytes to the HIV-1 protein, Tat resulted in the induction of PDGF at both the mRNA and protein levels. Furthermore, PDGF-BB induction was regulated by activation of ERK1/2 and JNK signaling pathways and the downstream transcription factor, early growth response 1 (Egr-1). Chromatin immunoprecipitation (ChIP) assays demonstrated binding of Egr-1 to the PDGF-B promoter. Exposure of astrocytes to PDGF-BB, in turn, led to both increased proliferation and release of pro-inflammatory

cytokines MCP-1 and IL-1 β . Since astrogliosis is linked to disease severity, understanding its regulation by PDGF-BB could aid in the development of therapeutic intervention strategies for HAND. Supported by MH-068212, DA020392, DA023397, DA024442, and DA0277729.

Norepinephrine Enhances the Vaginal Epithelial Immune Response to a Staphylococcal Superantigen. A. Brosnahan¹, B. Jones¹, L. Vulchanova¹, D. Brown¹; ¹Department of Veterinary and Biomedical Sciences, College of Veterinary Medicine, University of Minnesota, St. Paul, MN 55108.

Staphylococcus aureus is a Gram-positive pathogen that resides in the reproductive tract of approximately 20% of all women. It produces superantigen exotoxins which mediate toxic shock syndrome (TSS) by cross-linking antigen presenting cells and T cells to induce massive cytokine release. TSS toxin-1 (TSST-1) is the superantigen responsible for the majority of menstrual TSS cases. This toxin has pro-inflammatory effects in vaginal epithelial cells, and it is thought to induce migration of adaptive immune cells to the vaginal submucosa. We have previously reported [J. Neuroimmune Pharmacol., 5 (suppl. 1):S41, 2010] that cells immunoreactive for the norepinephrine (NE) transporter, a pharmacological target of cocaine, are present in human cervicovaginal mucosa and that NE delays wound healing in human vaginal epithelial cell (HVEC) monolayers. In this study, we tested the hypothesis that NE alters immune responses to TSST-1 in HVECs. Although it had no effect alone, 10 micromolar NE enhanced IL-8 release in response to TSST-1 (100 μ g/ml). Propranolol and the β 2-adrenergic receptor antagonist ICI 118551 inhibited this effect. Moreover, only the combination of TSST-1 and NE could induce a significant increase in intracellular cAMP levels, and this effect was also sensitive to ICI 118551. We are presently investigating the HVEC signaling pathways that mediate these effects. Psychostimulant drugs of abuse that act to increase NE levels may alter the ability of the vaginal mucosa to respond to pathogens and their associated exotoxins. Supported by NIDA DA-10200 and NIDA T32DA007097.

The Effects of Buprenorphine and CCL2 On Monocytes and the Blood Brain Barrier. L. Carvalho¹, L. Lopez¹, FY Che¹, J Lim¹, L Miller¹, L Weiss¹, RH Angeletti², JW Berman¹; ¹Department of Pathology, Albert Einstein College of Medicine, Bronx, NY 10461, ²Department of Developmental and Molecular Biology, Albert Einstein College of Medicine, Bronx, NY 10461, ³Microbiology and Immunology, Albert Einstein College of Medicine, Bronx, NY 10461.

HIV-1 enters the CNS, resulting in neurocognitive impairment in many infected people. Many HIV infected opiate abusers have increased inflammation that contributes to this impairment. Buprenorphine (bup) is used to treat opioid addiction. The effects of bup on transmigration on HIV infected monocytes (MON) across the Blood Brain Barrier (BBB) are unknown. We hypothesize that bup will alter tight junction proteins (TJP) on MON and on BBB to change the ability of HIV infected and uninfected MON to transmigrate into the CNS in response to CCL2. We showed that increased numbers of HIV infected MON cross the BBB in response to CCL2. We are examining the effects of bup and CCL2 on TJP of human MON and brain microvascular endothelial cells (BMVEC) necessary for MON to cross the BBB. We showed that CCL2 increased JAM-A and PECAM phosphorylation whereas CCL2+bup exhibited less increase in phosphorylation on MON. These phosphorylations are associated with MON migration. Thus bup could decrease neuroinflammation when CCL2 is increased in CNS HIV infection. Using proteomics, we quantified membrane peptides of MON and showed increased phosphorylation of leukosialin with bup+CCL2. Using BMVEC, CCL2 decreased JAM-A phosphorylation whereas CCL2+bup increased it. We found that CCL2 decreased total JAM-A and Claudin5 whereas CCL2 +bup showed baseline levels. As TJP are important in barrier integrity, bup could alter this integrity when CCL2 is increased in CNS HIV infection. Our data demonstrate some important mechanisms by which bup may impact NeuroAIDS. Supported by NIDA, Grant # 5P20DA026149-02 Einstein Proteomics Center for Study of the Neurological Consequences of HIV and Substance Abuse.

Protein Expression and Gene Regulation of CB2 in Human Immune Cells. J. Castaneda¹, SM Kiertscher², JD Roth², MD Roth^{1,2}, A Harui²; ¹Interdepartmental Program in Molecular Toxicology, University of California at Los Angeles, Los Angeles, CA 90026, ²Pulmonary and Critical Care Medicine, David Geffen School of Medicine at UCLA, Los Angeles, CA 90095-1690.

Cannabinoids, which represent the primary bioactive constituents of marijuana, are known to activate cannabinoid receptors CB1 and CB2 and interact with the endocannabinoid system. While these receptors are expressed in most organ systems, their distribution and function within the human immune

system is poorly understood. In order to gain greater insight into the role of the CB2 receptor in human immune function, we are characterizing its protein expression by peripheral blood mononuclear cells (PBMC) with flow cytometry (FACS), and RNA expression by quantitative real-time RT-PCR. Using a monoclonal anti-CB2 receptor antibody, reproducible expression of CB2 was routinely detected on the cell surface of fresh circulating human B cells (CD19+) from both non-smoking and marijuana-smoking subjects, but not on CD4+ T cells, CD8+ T cells, CD56+ NK cells, or CD14+ monocytes. In contrast, when cells were fixed and permeabilized to assess intracellular staining, CB2 expression was observed in all cell subsets. With 3-5 days of in-vitro culture, extracellular expression of the CB2 receptor on B cells was down-regulated, but intracellular expression was maintained on all cell subsets. Quantitative real-time RT-PCR detected CB2 gene expression at baseline in whole PBMC and in purified B cells, T cells and monocytes, which decreased with time in culture. This approach will allow us to evaluate the role of intra- and extracellular CB2 receptor expression and THC exposure in vitro and in marijuana smokers. Supported by NIH/NIDA Grants #R21-DA021813 and R01-DA03018.

Autophagy is Involved in the Neurovascular Toxicity of Nanoalumina. *L Chen*¹, B Hennig², M Toborek¹; ¹Neurosurgery, University of Kentucky, Lexington, KY 40503, ²College of Agriculture, University of Kentucky, Lexington, KY 40503.

Engineered nanomaterials (ENMs) have been widely used for various applications; however, their potential toxic effects are not fully understood. The current study focused on blood-brain barrier (BBB) disruption and neurovascular damage induced by treatment with one of the most abundantly manufactured ENMs, nanoalumina. Exposure of cultured human cerebral endothelial cells to nanoalumina (size 8-12 nm) elevated cellular oxidative stress, induced mitochondrial potential collapse, decreased tight junction expression, and cellular viability. Nanoalumina also induced activation of lysosomes and formation of autophagic vacuoles associated with elevated levels of autophagic marker proteins, p62 and LC-3. Delivery of nanoalumina into mouse cerebral circulation using a carotid surgical method altered expression of autophagy-related genes as detected by the brain DNA microarray. In addition, in situ immunostaining of brain slices detected time-dependent increases in LC-3 levels in different cell types of the neurovascular unit. Systemic treatment with nanoalumina elevated also autophagic activity in cerebral vessels, decreased tight junction expression, and elevated BBB permeability. Finally, exposure to nanoalumina increased brain infarct volume in mice subjected to a focal ischemic stroke model. Collectively, our study reveals that autophagy constitutes one of the critical mechanisms involved in nanoalumina-induced neurovascular toxicity in the central nervous system. Supported by American Heart Association Postdoctoral Scholarship, NIH ES 07380, DA027569, MH63022, MH072567, and NS39254.

Methamphetamine Reduces Influenza A Virus Replication in Human Lung Epithelia Cells. *YH Chen*¹, KL Wu¹, CH Chen¹; ¹Division of Mental Health and Substance Abuse, National Health Research Institutes, Miaoli County, Taiwan, 35053.

Methamphetamine is a highly addictive psychostimulant that is among the most widely abused illicit drugs, with an estimated 15-16 million users in the world. Several lines of evidence suggest that chronic methamphetamine abuse is the major factor for the increased risks of infections with human immunodeficiency virus and possibly other pathogens, due to the immunosuppressive property of this drug. Influenza A virus infections frequently cause epidemics and pandemics of respiratory disease. However, there is little known about whether methamphetamine has the ability to enhance influenza A virus replication, increasing susceptibility to infections in methamphetamine abusers. Herein, we investigated the effects of methamphetamine on influenza A virus replication in the human lung epithelia cell line A549. We report the first evidence that methamphetamine reduces expression and delays localization of viral proteins, resulting in a reduced viral propagation. The underlying mechanism(s) responsible for the action of methamphetamine on attenuating viral replication was explored and discussed.

Bone-Derived Mesenchymal Stem Cells (B-MSCs) Provided Protection Against Morphine-Induced Splenic and Thymic Cell Depletion. *K Cheng*¹, D Kumar¹, D Salhan¹, S Rehman¹, A Malhotra¹, S Gupta², P Singhal¹; ¹Feinstein Institute for Medical Research, Long Island Jewish Medical Center, New Hyde Park, NY 11040, ²Liver Center, Albert Einstein College of Medicine, Bronx, NY 10461.

Morphine has been reported to promote splenic and thymic atrophy. Usefulness of B-MSCs

transplantation in immune regulation and degenerative diseases is increasingly recognized. To better understand the effects of B-MSCs on morphine-induced immune suppression, we studied B-MSCs transplantation in morphine-treated mice. Donor B-MSCs were isolated from compact bone of male FVB/N mice. Cellular phenotype was verified by FACS for CD44, CD90, CD105, CD11b, CD34, CD86 and generation of adipocytes or chondrocytes. B-MSCs were labeled with ¹¹¹In-oxine. The biodistribution of B-MSCs were determined by gamma counting and verified by SRY PCR. B-MSCs localized mostly in lungs (76%), followed by liver (13%) and spleen (11%). B-MSCs were transplanted i.v. 24 hours after morphine pellet (75mg) implantation. Morphine pellet reduced splenic weight and decrease cellularity significantly at 1d (24%), but normalized by day 7. Thymus remained significantly smaller (30, 35 and 50% reduction at 1, 3 and 7d, respectively). B-MSCs transplantation partially protected morphine-induced thymic weight reduction. Moreover, spleen size returned to normal at 1d accompanied with restoring of cell content. MTT assay demonstrated that the proliferation of B-MSCs was not inhibited by morphine at 1 and 3d. In conclusion, B-MSCs were able to localize to specific organs via i.v. delivery. The immunosuppressive effect induced by morphine could be partially inhibited by transplantation of B-MSCs. Further studies of B-MSCs on immune regulation will be helpful for therapeutic strategies in opiate addicts.

Lipopolysaccharide Potentiates PCB-Induced Disruption of the Integrity of Brain Endothelial Cells.

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Polychlorinated biphenyls (PCBs) are worldwide spread environmental toxicants that are associated with numerous adverse health effects in humans. We reported that oral administration of individual PCB congeners (PCB153, PCB118, or PCB126; 150 µM/kg) to C57Bl/6 mice can disrupt the barrier function of intestinal epithelium. This effect was associated with translocation of lipopolysaccharide (LPS) from the intestine into the blood, resulting in a significant increase in plasma LPS levels 24 h post PCB treatment. In the present study, we evaluated whether LPS can contribute to cerebrovascular toxicity of PCBs using cultured brain endothelial cells (hCMEC/D3 cell line). A combined treatment with PCB118 (2 µM) and LPS (2 ng/ml) for 24 h reduced to a higher extent the levels of tight junction proteins occludin and ZO-2 as compared to exposure to PCB118 or LPS alone. These effects were associated with increased permeability to FITC-labeled dextran (20 kDa). Compared to the effects of PCB118 or LPS alone, a combined treatment also resulted in a higher activation of transcriptional factor IRF-3, suggesting stimulation of toll-like receptor pathways. These data support the hypothesis that LPS may be a contributing factor in PCB-induced dysfunction of the brain endothelium. Supported by ES 07380, DA027569, MH63022, MH072567, and NS39254.

Astrocytic Nef Expression in Sprague Dawley Rats Impairs Spatial Memory. *G Chompre¹, E Cruz², L Maldonado¹, JT Porter², RJ Noel¹*; ¹Biochemistry Department, Ponce School of Medicine, Ponce, PR 00731, ²Pharmacology and Physiology Department, Ponce School of Medicine, Ponce, PR 00731.

Even with antiretroviral therapy, people with HIV associated neurocognitive disorders (HAND) suffer from memory impairment such as remembering to take medication. HIV infects several cell types in the brain including microglia and astrocytes. Astrocytes are non-productively infected by HIV since they only support production of viral proteins such as Nef. Nef is found to be highly expressed in astrocytes in post-mortem brain tissues from patients with HIV associated dementia (HAD) and rhesus macaques infected with SIV. We tested if unilateral infusion of astrocytes expressing Nef into the hippocampus produces memory loss in Sprague Dawley rats. To test the hypothesis, we delivered a Nef gene into astrocytes and infused the cells into the right hippocampus of rats. After recovery, novel location & object recognition were performed. Rats implanted with astrocytes expressing Nef showed impaired novel location recognition in comparison with controls. Nef exposed rats showed a reduced number of neurons in the CA3 field. These results suggest that even unilateral delivery of Nef is sufficient to impair spatial memory and may play a role in neuronal loss producing HAND. Supported by RR03050 and R25GM082406.

Morphine Withdrawal Stress Synergized with Corticosterone to Inhibit IL-12p40 Expression by Hyperactivating ERK1/2. *S Das¹, S Roy¹*; ¹Department of Surgery, University of Minnesota, Minneapolis, MN 55455, ²Department of Pharmacology, University of Minnesota, Minneapolis, MN 55455.

Very few studies have examined the effects of morphine-withdrawal (MW) on immune functioning, and

of these even fewer describe the mechanisms underlying withdrawal induced immunosuppression. Although, stress has been well documented to be immunosuppressive, the role of corticosterones in MW induced immunomodulation has not been investigated. This study explores the role of corticosterone in MW induced suppression of IL-12p40, a major soluble mediator playing a pivotal role in immunoprotection. Using WT and Mu opioid receptor knock-out mice and a murine alveolar macrophage cell line, we show that MW significantly reduced LPS induced IL-12p40 promoter activity, mRNA levels and protein expression both in vivo and in vitro. This suppression is due to decreased consensus binding of transcription factors NF- κ B, AP-1 and C/EBP to IL-12p40 protein. MW effects were abolished in the MORKO mice. While corticosterones alone do not significantly modulate these transcription factors, in combination with MW a synergistic reduction was observed. The synergistic effects were non-genomic since nuclear translocation of glucocorticoid receptor was not significantly different between MW and corticosterone treatment. In contrast, in MW samples in the presence of LPS and corticosterone, a significant hyperactivation of ERK1/2 MAPK was observed while other MAPKs are inhibited. These data clearly indicate a synergistic role for corticosterones in morphine-withdrawal induced inhibition of IL-12p40 production through a mechanism that involves ERK1/2 hyperactivation. Supported by R01DA12104, R01DA022935, K02DA015349, and P50DA11806.

Neuronal MicroRNA-142 is Up-Regulated in HIVE/SIVE and Inhibits the Expression of Key Proteins. *A Datta Chaudhuri*¹, *SV Yelamanchili*¹, *HS Fox*¹; ¹Pharmacology and Experimental Neuroscience, University of Nebraska Medical Center, Omaha, NE 68198.

Human immunodeficiency virus-1 (HIV-1)-associated neurocognitive disorder (HAND) and the pathological entity of HIV encephalitis (HIVE) are important complications of HIV infection. Our understanding of upstream factors driving alterations in gene and protein expression in HAND is nascent. Recently microRNAs (miRs), small non-coding RNAs fine-tuning gene expression, have been implicated in many neurodegenerative diseases. We carried out miR profiling of the brains of rhesus macaques suffering from simian immunodeficiency virus encephalitis (SIVE, the rhesus equivalent of HIVE) and of HIVE patients. In addition to miR-21 (Yelemanchili, Datta Chaudhuri et al, 2010), we found that miR-142-3p and -5p are up-regulated in the caudate nucleus and hippocampus in infected monkeys and humans. We hypothesize that miR-142, by inhibiting expression of key neuronal proteins, contributes to neuronal dysfunction in HIVE. Combined fluorescent in situ hybridization and immunofluorescence labeling demonstrated that miR-142 co-localizes with neuronal marker MAP2. To determine miR-142 targets, gene expression was compared in neuroblastoma cell lines transduced to stably express miR-142 or a scrambled miR. Microarray analysis indicated significant down-regulation of 61 mRNAs in miR-142 expressing cells. Quantitative proteomics using SILAC revealed significant down-regulation of 28 proteins. DAVID pathway analysis showed enrichment of key functional molecules. Validation of miR-142 targets in vitro and in HIVE/SIVE brain is underway to reveal mechanism of neuronal dysfunction in this disease. Supported by NIMH, MH062261.

Cocaine Increases Platelet-Derived Growth Factor Expression in Human Brain Microvessel Endothelial Cells Through Notch-1 Signaling. *MD Duan*¹, *HY Yao*¹, *SB Buch*¹; ¹Department of Pharmacology and Experimental Neuroscience, University of Nebraska Medical Center, Omaha, NE 68198.

Neuroinflammation associated with advanced HIV-1 infection is often exacerbated in cocaine-abusing, HIV-infected individuals. The underlying mechanisms could, in part, be attributed to the increased impairment of blood brain barrier integrity in the presence of cocaine. Platelet-Derived Growth Factor-BB (PDGF-BB) has been implicated in a number of pathological conditions, specifically attributable to its potent mitogenic effects. Its modulation by drug abuse, however, has received very little attention. In the present study, we demonstrated cocaine-mediated induction of PDGF-BB and Notch1 in human brain microvascular endothelial cells. In this study, we link Notch1 signaling to PDGF-BB, and show that PDGF-BB is a novel immediate Notch target gene. PDGF-BB expression induced by cocaine was abrogated by gamma secretase inhibitor-DAPT. Furthermore, over-expression of intracellular domain of Notch1 significantly increased PDGF-BB expression. Functional implication of up-regulated PDGF-BB as a vascular permeant was confirmed in cell permeability and transmigration assays. In vivo relevance of these findings were further corroborated in cocaine-treated mice that were administered DAPT (i.p. 40mg/kg). Cocaine exposure resulted in increased permeability of the endothelial barrier and this effect was abrogated in mice exposed to DAPT, thus underscoring its role as a vascular permeates.

Understanding the regulation of PDGF-BB expression may provide insights into the development of potential therapeutic targets for neuroinflammation associated with HIV infection and drug abuse.

Differential Migration of Ly6C+ (Monocytes) and CD3+ T Cells Following Morphine and Tat Exposure. *R Dutta*¹, R Charboneau², H Yu¹, J Meng¹, R Barke², S Roy¹; ¹Department of Surgery, University of Minnesota, Minneapolis, MN 55455, ²Department of Surgery, Veterans Affairs Medical Center, Minneapolis, MN 55417.

HIV affects the host nervous system by producing distinct neurological complications or by causing immunodeficiency with susceptibility to opportunistic infections. Incidence associated with this condition is more profound in opiate drug users. Mechanism associated with the immunopathogenesis of this co-infection among morphine (MS) drug abusers is poorly understood. We delineate peripheral leukocyte subsets and immunoregulatory factors present in the brains of *S. pneumoniae* infected wild type (wt) and tat-transgenic (tat -tg) mice following chronic MS exposure. In vivo imaging showed significantly greater bacterial dissemination in MS treated mice in contrast to controls. FACS data showed a differential migration pattern of Ly6C+ and CD3+ cells in MS treated tat-tg compared to wt. Morphine treatment in wt resulted in a 5 fold increase in Ly6C+ cells compared to placebo but no significant difference was observed between MS treated tat-tg compared to MS treated wt. Morphine treatment resulted in a marginal increase in CD3+, however in tat-tg mice the migration was 3 fold higher than wt. Results suggest that MS and tat recruit differential immune cells to the CNS and the migration of CD3+ cells is synergistic. Significant increase in CCR5 expression on CD3+ cells was observed on MS treated mice. CD45 low CD11b+ and CD45 high CD11b+ cells showed 1.2 & 2 fold increase in CCR5 expression in mice brain treated with MS, respectively. Thus differential trafficking of Ly6C+ and CD3+ cells may be responsible for the neuropathogenesis observed in opiate drug abusers having co-infection. Supported by RO1 DA 12104; RO1 DA 022935; KO2 DA.

Amelioration of HIV-1 Tat-Induced Neuronal Injury by Phytoestrogens. *V Espensen-Sturges*¹, S Bertrand¹, MV Aksenova¹, MY Aksenov¹, S Adams¹, CF Mactutus¹, RM Booze¹; ¹Department of Psychology, University of South Carolina, Columbia, SC 29208.

Development of new strategies to prevent the neurobehavioral abnormalities associated with chronic HIV-1 infection is critical to the advancement of care of these patients. Our previous studies have indicated that estrogen and phytoestrogenic compounds, namely genistein and diadzein, may offer neuronal protection against HIV-1 Tat-initiated mitochondrial apoptosis via an estrogen receptor (ER-beta)-dependent mechanism. In this study, dose-response curves of neuroprotection provided by genistein and diadzein were generated. At the low concentration of 0.1 μ M, both genistein and diadzein provided neuroprotection (85% or higher) against Tat (50 μ M) toxicity. In addition, we found cytomorphological evidence for the ability of different phytoestrogens to ameliorate degeneration and promote rebuilding of neuronal dendrites following HIV protein (Tat)-mediated neurotoxic insults. Supported by University of South Carolina Research Foundation Grant, NIH Grant DA013137, and University of South Carolina Magellan Scholars.

Human Astrocytes Regulate miRNA Expression During Neuroinflammation. *JA Fields*¹, A Ghorpade¹; ¹Cell Biology and Anatomy, University of North Texas Health Science Center, Fort Worth, TX 76107.

Astrocytes are known to be affected by, and reactive to neuroinflammation, but the molecular mechanisms leading to changes in gene expression are not well understood. MicroRNA (miRNA) represents small noncoding RNA molecules that are transcriptionally regulated and are capable of influencing translation of mRNA. It is thought that a single miRNA can regulate many genes and one or more of these RNA species regulates most genes. A growing body of evidence supports the assertion that bystander effects on astrocytes lead to complications in HIV-associated neurocognitive disorders (HAND) and miRNA may contribute to regulation of gene expression during inflammation. In this work, we hypothesized that during neuroinflammation human astrocytes regulate miRNA that in turn regulate gene expression, ultimately contributing to disease process. We treated human astrocytes with IL-1 β for eight hours, isolated total RNA and then analyzed expression of 846 human miRNA using an Affymetrix miRNA microarray chip. Statistical analyses revealed 16 miRNA that were significantly altered as compared to untreated astrocytes; 12 were upregulated and 4 were downregulated. Of these, miR-155 was the most increased and miR-432 the most decreased. Expression levels of the 16 miRNA were confirmed by

quantitative polymerase chain reaction. Identification of miRNA that are regulated by astrocytes during neuroinflammation may lead to novel insights as to how these cells change during HAND.

Morphine and HIV-1 Tat-Dependent Synaptodendritic Injury in Striatum: Focal Increases in Ca²⁺ and Differential Activation by NMDA and AMPA Receptors. *S Fitting*¹, *S Zou*¹, *PE Knapp*¹, *KF Hauser*¹; ¹School of Medicine, Virginia Commonwealth University, Richmond, VA 23298.

Previous studies found correlative relationships between synaptodendritic injury and neurobehavioral defects with combined HIV-1 Tat and morphine exposure in the absence of neuron death. Tat induction or morphine treatment can disrupt neuronal synaptic organization (as assessed in Golgi-impregnations and by electron microscopy) and coordination/sensorimotor activity; however, in combination there were synergistic dendritic pathology including the formation of bead-like varicosities and synaptodendritic degeneration. In vitro studies revealed nearly identical dendritic swellings and degenerative changes as seen in vivo, which are hypothesized to be caused by focal excitotoxic injury influx of Na⁺, ATP depletion and energetic compromise, and Ca²⁺ overload. Chronic morphine has been shown by others to downregulate surface AMPA receptors at synaptic and extrasynaptic sites and modify neuronal activity, which significantly affects glutamatergic signaling. Importantly, Tat ± morphine caused significant focal increases in Ca²⁺ along the dendrite that coincided with the dendritic varicosities. Moreover, Tat ± morphine-dependent losses in ion homeostasis and dendritic swelling were attenuated by NMDA, while AMPA receptor antagonists are currently being explored. The convergence of Tat and morphine-driven dysregulation of excitotoxic glutamatergic signaling, and the accompanying losses in ion homeostasis, appear to be important sites of opioid-HIV-1 Tat synergistic interactions and is likely to be critical in underlying sublethal reductions in synaptic connectivity and dendritic injury. Supported by NIDA P01 DA18633 and R01 DA19398.

Molecular Mechanism of Immunosuppression by Cocaine In HIV-1 Infected Subjects: Role of TLR3. *N Gandhi*¹, *M Agudelo*¹, *ZM Saiyed*¹, *Y Adriana*¹, *C Spadola*¹, *VB Pichili*¹, *T Samikkannu*¹, *N Jesica*¹, *P Khatavkar*¹, *MN Nair*¹; ¹Department of Immunology/ INIP, College of Medicine/Florida International University, Miami, FL 33199.

Cocaine dependence is associated with potent immunosuppression and an increased risk for HIV-1 infection. The innate immune response mediated by Toll-like receptors (TLRs) activates multiple intracellular pathways to release pro- and anti-inflammatory cytokines, chemokines and antiviral factors. TLR3 recognizes double stranded RNA leading to activation of Interferon Regulatory Factor (IRF) -3 and -7 to induce antiviral response by IFNs. Although the role of TLR3 in HIV-1 infection is known, the effect of cocaine on TLR3 expression in HIV-1 infection is not clearly elucidated. Since dendritic cells are the first line of defense against HIV-1 infection, we studied the expression of TLR3 in monocyte-derived dendritic cells (MDDC) from HIV-1 infected cocaine users. Our results indicate that TLR3 expression was significantly downregulated in HIV-1 infected cocaine users compared to HIV-1 infected non cocaine users. These findings were further confirmed in an in vitro HIV-1 infection model and found that HIV-1 induced upregulation of TLR3 gene and protein expression was significantly lower in MDDC infected with HIV-1 and treated with cocaine compared to MDDC infected with HIV-1 only. Further, the expression of downstream signaling molecules, IRF-3 and IRF-7 as well as effector molecule, IFN-γ were significantly downregulated in HIV-1 infected cocaine treated MDDC. Thus, our results suggest that cocaine may downregulate innate immune response in HIV-1 infected subjects by TLR-3 mediated mechanism, involving dysregulation of pro and anti-inflammatory molecules. Supported by NIMH and NIDA.

Morphine Enhances HIV-1 Entry into Kidney Cells Through a Novel Pathway. *HG Goel*¹, *P Singh*¹, *A Malhotra*¹, *M Husain*¹, *P Singhal*¹; ¹Department of Nephrology, North Shore-Long Island Jewish Health System, Great Neck, NY 11021.

Drug addicts are considered to have increased risk for the development of HIV-associated nephropathy (HIVAN). Renal biopsy data suggest the role of HIV-1 infection of kidney cells in the development of HIVAN. However, the mode of HIV-1 entry in kidney cell is not clear. Kidney cells do not carry conventional HIV-1 receptors. Therefore, to determine the entry of HIV-1 in kidney cells, lymphocytes (LY) with or without HIV-1 infection (HIV-LY) were co-cultivated with tubular cells. FACS analysis showed the population of CD4+ve T cells dropped significantly in these experiments. HIV-LY had even higher apoptosis and resulted in enhanced tubular cell HIV-1 expression. Morphine treatment not only enhanced T cell apoptosis but also displayed enhanced tubular cell HIV-1 expression. Interestingly,

morphine-treated tubular cells also displayed up regulated PD-L1 expression. On the other hand, pretreatment of tubular cells with anti-PD-L1 antibody attenuated T cell apoptosis, and thus indicating the role of PD-1:PD-L1 pathway. Imaging studies (acridine orange, double labeling of tubular cells and T cells, and electron microscopy) displayed tubular cells containing T cell fragments. Since both, naloxone and cytochalasin B prevented morphine induced tubular cell HIV-1 expression, it appeared that both opiate receptors and endocytic pathway contributed to T cell uptake. Since HIV-1 did not induce productive infection in tubular cells directly, it appears that phagocytosed apoptosed T cells provided a suitable milieu for productive HIV-1 infection. Supported by National Institute of Health.

Ethanol-Induced Upregulation of IL-6 and IL-8 in Astrocytes is Mediated by the NF- κ B Pathway. *R Gupte*¹, *A Shah*¹, *S Kumar*¹, *A Kumar*¹; ¹Division of Pharmacology and Toxicology, University of Missouri-Kansas City, Kansas City, MO 64108.

Alcohol abuse has been implicated as the cause of various health problems all over the world. Alcohol is known to be toxic to the brain but the mechanisms contributing to neurotoxicity have not been fully elucidated. In this study, we measured the induction of the pro-inflammatory cytokines IL-6 and IL-8 in a human fetal astrocyte cell line SVGA after treatment with ethanol. Both IL-6 and IL-8 mRNA levels increased in a time-dependent manner after treatment with a single dose of 50mM ethanol. Peak induction of both cytokines was observed at 3 h (21.5 fold \pm 2.93 for IL-6 and 42.76 \pm 3.02 for IL-8) and expression levels of these cytokines declined to levels below that of the control at 12 h and 24 h. To determine if these effects were mediated by the NF- κ B pathway, time-dependent activation of NF- κ B was studied. Nuclear levels of p50 were significantly higher as compared to cytoplasmic levels after ethanol treatment and maximum translocation was observed 1 h post treatment. Pre-treatment with I- κ B kinase inhibitor SC-514 abrogated the ethanol-mediated upregulation of IL-6 and IL-8 in a concentration-dependent manner with 50 μ M dose showing maximum inhibition. These results demonstrate that NF- κ B plays a key role in ethanol-mediated upregulation of inflammatory cytokines in the brain. These studies will further be corroborated with the use of siRNA against NF- κ B to block the induction of released cytokines IL-6 and IL-8. Furthermore, these results suggest that the NF- κ B pathway is a potential therapeutic target for treatment of ethanol-induced neuroinflammation.

Interactive Effects of HIV-1 Tat and Opiates on CNS Progenitor Cells. *YK Hahn*¹, *CM Bull*¹, *S Fitting*², *P Vo*¹, *KF Hauser*², *PE Knapp*¹; ¹Departments of Anatomy & Neurobiology, Virginia Commonwealth University, Richmond, VA 23298, ²Departments of Pharmacology & Toxicology, Virginia Commonwealth University, Richmond, VA 23298.

Injection drug abuse and HIV infection are interlinked epidemics, and HIV patients with a history of opiate abuse appear to have higher incidence of neurologic dysfunction. We hypothesize that neuron/glia progenitors (NPC/GPCs) may be targets of HIV-1/viral proteins and opiate drugs of abuse, leading to effects on gliosis, inflammation and CNS repair. Previous work showed that HIV-1 Tat, but not gp120, altered progenitor chemokine secretion (Hahn et al., *J Neurochem*, 2010). Here we report that Tat \pm opiate exposure profoundly affects progenitor cell motility/migration, proliferation and lineage progression in vitro. Tat exposure reduced proliferation (BrdU), first in Sox2+, and then in Olig2+ populations, but only when the cells were co-exposed to morphine. Using an inducible transgenic mouse, we examined Tat effects in vivo. In adult mice, similar to in vitro findings, Tat expression reduced overall proliferation (Ki67+) in the striatum, largely through effects on Olig2+ cells. Such effects likely led to the reduced numbers of oligodendrocytes (OLs) that we observed in the corpus callosum. Based on reports of gender differences in OL populations, we tested gender specific responses to HIV-1 Tat in vivo. Although OLs and NeuN+ neurons in males and females showed similar responses to Tat \pm opiates, male mice with chronic Tat induction (3 mo) performed significantly worse than female mice in a behavioral study (rotarod). Males also had significantly increased numbers of 3-nitrotyrosine+ microglia, suggesting that their behavioral deficits may relate to enhanced inflammation. Supported by DA024461 and NS069216.

CB2-Selective Cannabinoids Directly Inhibit T-Cell Function in the Mixed Lymphocyte Reaction (MLR). *RR Hartzell*¹, *JJ Meissler*¹, *MW Adler*¹, *TK Eisenstein*¹; ¹Center for Substance Abuse Research, Temple University School of Medicine, Philadelphia, PA 19140, ²Department of Microbiology and Immunology, Temple University School of Medicine, Philadelphia, PA 19140.

The MLR is considered to be an in vitro correlate of skin and organ transplant rejection. In this assay, spleen cells from two different mouse strains are incubated together. The T-cells of one strain respond to

the foreign cells by proliferating. We have previously shown that the CB2-selective agonists JWH-015 and O-1966 inhibit the MLR when added to unfractionated murine spleen cells. To corroborate whether T-cell function was decreased by CB2-selective agonists, IL-2 release was quantitated by ELISA using MLR culture supernatants of unfractionated spleen cells. It was found that both CB2 agonists inhibited IL-2 release in a dose-dependent manner, indicating that the cannabinoids inhibit this parameter of T-cell function. To test whether the cannabinoids act directly on the T-cells or via effects on accessory cells, murine splenocytes were separated into highly purified subpopulations using flow cytometry. Specifically, CD3+ (T-cells) and CD11b+ (myeloid derived cells) fractions were sorted and individually treated with JWH-015 or O-1966 before being added back to the remainder of the spleen cells, which were either CD3 or CD11b depleted. Inhibition of the MLR was observed only when the CD3+ population was treated with a cannabinoid, and not when the CD11b+ population was treated, indicating that the CB2 agonists act directly on T-cells. These results elucidate the mechanism of CB2 compounds in the MLR, and provide additional support for the potential of CB2-selective cannabinoids as immunosuppressive agents that may be useful therapies to block graft rejection. Supported by NIDA grants DA13429, DA06650, and T32-DA07237.

Cytokine, Chemokine and their Receptors' Expression on Exposure to Endotoxin in the HIV-1 Transgenic Rat. *N Homji-Mishra*¹, X Mao¹, EF Langsdorf¹, SL Chang^{1,2} ¹Institute of NeuroImmune Pharmacology, Seton Hall University, South Orange, NJ 07079, ²Department of Biological Science, Seton Hall University, South Orange, NJ 07079.

Prior exposure to endotoxin renders innate immune cells to become refractory to subsequent endotoxin challenge in endotoxin-tolerance (ET). HIV-1 affects the immune system and thus it is clinically relevant to study ET during HIV-1 infection. In this study, the gene-expression of 84 cytokines, chemokines and their receptors, in the brain, spleen and serum were studied in lipopolysaccharide (LPS) treated rats. We also studied the protein levels of 7 of these 84 genes. HIV-1 transgenic (HIV-1Tg) rats (animal-model for HIV-1 infection controlled by HAART) and control animals, F344 rats (n=12 ea) were randomly treated with 2 doses of 250 µg/kg LPS (LL) or saline (SS) and then with a lethal dose of 5 mg/kg LPS (+L) or saline (+S). Tissues and blood were collected 2 hours post-last-dose. We found that the same cytokine and chemokine genes in brain (21) and in spleen (41) were modulated ≥ 2 folds in both strains of rats. However, the magnitude of modulation of most genes for the same LPS treated group was different in F344 versus HIV-1Tg rat brain or spleen. Pro- and anti-inflammatory cytokines were most up-regulated in SS+L group followed by LL+L group compared to control (SS+S) suggesting that the animals in LL+L group were in an ET state. These results give a systemic picture of the cytokine/chemokine profiles and a clearer understanding of the neuroimmune (brain and spleen) response during ET. The differences between F344 and HIV-1Tg cytokine/chemokine profiles show that the presence of HIV-1 viral proteins may alter the expression profile of inflammatory molecules during ET. Supported by NIH DA007058 & DA016149 to SLC.

HIV-1 Transgenic Rats: Alterations in the Preattentive Process of Sensorimotor Gating. *LL Hord*¹, KM Webb¹, LM Moran¹, CF Mactutus¹, RM Booze¹; ¹Behavioral Neuroscience Program, University of South Carolina, Columbia, SC 29223.

With the great success of combination antiretroviral therapy (cART) regimens, AIDS has transitioned from an acute disease with rapid loss due to AIDS-related mortality to a chronic neurological disease with normal longevity. Despite undetectable HIV-1 infection under cART, HIV-1 associated neurological disorders (HAND) occur in more than 50% of individuals. In the present study we utilized the Sprague-Dawley (SD) HIV-1 transgenic (HIV-1 Tg), which expresses 7 of the 9 HIV-1 genes, to examine the preattentive process of sensorimotor gating. SD HIV-1 Tg rats and non-transgenic controls (ns=11) were obtained from the supplier (HSD) and studied beginning at 90 days of age. At three monthly intervals, all animals were individually tested for their auditory startle response (ASR) and prepulse inhibition (PPI) of the ASR. Each animal was tested using an acoustic startling stimulus [100dB(A) broad-band noise stimulus] and an acoustic prepulse [85 dB(A) broad-band noise stimulus] in a sound attenuating double-wall chamber with a continuous white noise 70dB(A) background. The protocol used a 5-min acclimation period, 6 ASR trials, and 36 PPI trials [ISIs of 0, 8, 40, 80, 120, and 4000 ms, 6-trial blocks, Latin square design]. Alterations in the ASR and PPI were observed at all time points, suggesting the HIV-1 Tg rat develops long-term alterations in the preattentive process of sensorimotor gating, despite its aviremic state. Such subtle and early-detected cognitive alterations may have significant utility in predicting

progression of central nervous system disease markers. Supported by NIH grants: DA13137 and HD043680.

MIR-29 Regulates Morphine and HIV Protein-Decreased Platelet-Derived Growth Factor

Expression. *GH Hu*¹, SB Buch¹; ¹Department of Pharmacology and Experimental Neuroscience, University of Nebraska Medical Center, Omaha, NE 68198-5880.

Extensive neuronal damage and loss is a hallmark of HIV-associated neurological disorders (HAND), a term used to describe the range of cognitive and motor impairments that occur in individuals suffering from AIDS. Opiate drug abuse accelerates the incidence and progression of HAND, however, mechanisms underlying the ability of these drugs to enhance the pathological effects of HIV-1 in the brain remains elusive. Morphine is often abused by HIV-1 infected patients. Platelet-derived growth factor-BB (PDGF-BB) has been reported to provide tropic support for neurons in the central nervous system. In the present study, it was demonstrated that PDGF-BB expression was decreased in basal ganglia in virus (SIV)-infected macaques with or without chronic morphine administration with concomitant increased miR-29b expression compared with the saline control group. In vitro relevance of this finding was further corroborated in conditioned medium from morphine and HIV-1 protein Tat treated A172-astrocyte cell line, which decreased PDGF-BB and increased miR-29b expression with concomitant decreased cell viability. Furthermore, it was shown that PDGF-BB was a target for miRNA-29b as evidenced that binding of miR-29 to the 3'- untranslated region of PDGF-B mRNA results in translational repression in SH-SY5Y cells. Understanding the regulation of PDGF-BB expression may provide insight into the development of potential therapeutic targets for neuronal loss in HIV-1 infection with morphine drug abuse.

PPAR- α and PPAR- γ Agonists Differentially Protect Against HIV Tat-Induced Alterations of Claudin-5 Expression and Activation of Redox Signaling in FVB/NJ Mice.

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Tight junctions (TJs) are the main structural and functional elements that regulate the blood-brain barrier (BBB) integrity. In the present study, we investigated the mechanisms of peroxisome proliferator-activated receptor (PPAR)-mediated protection against HIV Tat-induced alterations of the BBB integrity and claudin-5-expression in vivo. Delivery of HIV-1 Tat via the internal carotid artery induced transmicrovessel migration of monocytes and increased the BBB permeability in wild-type FVB/NJ mice; however, these effects were attenuated in mice treated with fenofibrate (PPAR- α agonist, 100 mg/kg) or rosiglitazone (PPAR- γ agonist, 10 mg/kg) once daily for 1 week. Surprisingly, the baseline expression of claudin-5 was lower and treatment with HIV-Tat induced disruption of the BBB integrity to a higher extent in matrix metalloproteinase (MMP)-9 knockout mice as compared to wild-type controls. Exposure to HIV-1 Tat decreased claudin-5 levels and activated ERK1/2 and Akt in the microvessels of wild-type controls. These effects were attenuated by co-treatment with fenofibrate but not by rosiglitazone. In contrast, ERK1/2 and Akt were not affected by treatment with Tat or PPAR agonists in MMP-9 deficient mice. The results of the present study indicate that HIV-1-Tat-induced alterations of TJ protein expression and redox-regulated signaling proteins are differentially regulated by PPAR- α and PPAR- γ agonists depending upon MMP-9 activity. Supported by in part DA027569, MH63022, MH072567, NS39254 and AHA09POST2060217.

The Role of Cytochrome P450 on Alcohol/Nicotine-Mediated Oxidative Stress in U937

Macrophages. *M Jin*¹, R Earla¹, AK Shah¹, R Gupte¹, AK Mitra¹, A Kumar¹, S Kumar¹; ¹School of Pharmacy, University of Missouri-Kansas City, Kansas City, MO 64108.

Substance abuse, especially alcohol and tobacco, is prevalent among HIV-1 infected individuals. Cytochrome P450 (CYP) enzymes are involved in the metabolism of alcohol (CYP2A6, CYP2E1) and nicotine (CYP2A6), both of which are known to cause oxidative stress in the liver. There are some reports that alcohol- and nicotine-induced oxidative stress increases HIV-1 replication. However, the mechanism by which they increase oxidative stress and HIV-1 replication is not known. This study is designed to explore the role of CYPs in alcohol- and nicotine-mediated oxidative stress in U937 macrophages. We analyzed the expression levels of CYPs and determined the levels of oxidative stress in acutely alcohol- and nicotine-treated U937 macrophages. Alcohol (100 mM) showed significant induction of CYP2A6 and CYP2E1 compared to the control. Furthermore, alcohol increased the production of reactive oxygen species (ROS), a marker of oxidative stress. 1 μ M nicotine significantly increased CYP2A6 expression

and ROS production, which was consistent with nicotine metabolism. Furthermore, the CYP2A6-selective inhibitor, tryptamine completely blocked the production of ROS, suggesting the involvement of CYP2A6 in nicotine-mediated ROS production. Overall the results demonstrate the role of CYP2A6 and CYP2E1 on alcohol/nicotine-mediated oxidative stress in U937 macrophages. We are now in the process of examining the acute and chronic effects of the combination of alcohol and nicotine on CYPs-mediated oxidative stress and their impact on HIV-1 replication. Supported by University of Missouri Research Board Grant.

Chemokine CXCL8 is Regulated in Activated Astrocytes through MAPKs and SHP-2. *M Kaur*¹, L Tang¹, K Borgmann¹, A Ghorpade¹; ¹Department of Cell Biology and Anatomy, University of North Texas Health Science Center, Fort Worth, TX 76107.

CXCL8 is an important chemokine implicated in HIV-associated dementia (HAD). Interleukin (IL)-1-beta activated astrocytes exhibit significant upregulation of CXCL8, and the mechanisms involved are being elucidated. In the present study, we demonstrate that astrocytes produce CXCL8 in Mitogen activated protein kinase (MAPK) and Src homology domain 2 protein tyrosine phosphatase (SHP-2) dependent mechanism. MAPKs are family of kinases that transduce variety of external signals and SHP-2 is implicated to act upstream of MAPKs. Primary human astrocytes were treated with or without MAPK (p38-SB203580; JNK-SP600125; and ERK-U0126) and SHP-2 inhibitors (sodium orthovanadate and PHPs1), followed by activation with IL-1 β . Brain tissues from HIV infected individuals were also analyzed for CXCL8 levels, compared to age matched controls. Astrocytes were transfected with SHP-2 overexpression and dominant negative control plasmids. Changes in mRNA and protein levels were analyzed by real-time PCR, ELISA and western blot analysis to establish the role of these signaling pathways in astrocyte CXCL8 regulation. Significant downregulation ($p < 0.001$) in IL-1 β -mediated CXCL8 mRNA and protein expression was found in astrocytes pre-treated with MAPK and SHP2 inhibitors. Elevated levels of CXCL8 mRNA and protein were found in brain lysates of HIV infected individuals. Overexpressing SHP-2 in astrocytes resulted in higher CXCL8 levels. These results indicate that the proinflammatory chemokine CXCL8 upregulation in activated astrocytes is MAPK and SHP-2 dependent. Supported by NIH/NIMH/1RO1MH087345-01.

Tumor Necrosis Factor- α Enhances Astroglialogenesis and Inhibits Neurogenesis of Human Fetal Neural Progenitor Cells through JNK/STAT3 Pathway. *X Lan*¹, H Peng¹, J Zheng¹; ¹Laboratory of Neuroimmunology and Regenerative Therapy, University of Nebraska Medical Center, Omaha, NE 68198.

Active neurogenesis, which relies upon the proliferation, migration, and differentiation of neural progenitor cells (NPC), occurs throughout life and is very important for the development and the well-being of the brain. Impaired neurogenesis has been suggested to add to the woe of neurodegenerative disorders such as HIV-1 associated neurocognitive disorders (HAND). Our previous studies demonstrated that HIV-1-infected and LPS-activated monocyte-derived macrophages inhibit neurogenesis while enhance astroglialogenesis through secretion of tumor necrosis factor- α (TNF- α) and activation of transcription factor signal transducers and activator of transcription 3 (STAT3). Here we further test the hypothesis that TNF- α -mediated astroglialogenesis is through the JNK/STAT3 pathway. Fetal neural progenitor cells were treated with recombinant TNF- α together with or without c-Jun N-terminal Kinase (JNK) inhibitor SP600125 in the differentiation medium. Results showed that TNF- α dramatically activated JNK and STAT3, and also significantly enhanced astroglialogenesis. Both the STAT3 activation and enhanced astroglialogenesis were abrogated by the addition of SP600125. These observations demonstrated that TNF- α could enhance astroglialogenesis and/or inhibit neurogenesis through the JNK/STAT3 pathway. This study generates important data elucidating the role of TNF- α in neurogenesis and may provide insight into new therapeutic strategies for brain inflammation. Supported by the National Institutes of Health: R21 NS 066841 to HP, R01 NS041858, R01 NS061642 and R01 NS043985 to JZ.

Negative Regulation of Neuronal Differentiation by TLR4 Activation. *JL Li*¹, L Ye¹, X Wang¹, YZ Wang¹, Y Persidsky¹, WZ Ho¹; ¹Department of Pathology, School of Medicine, Temple University, Philadelphia, PA 19140.

Neurogenesis is the process by which functionally integrated neurons are generated from neural stem cells (NSCs). However, the mechanisms underlying the regulation of neurogenesis are largely unknown.

In this study, we examine the role of Toll-like receptors (TLRs), a family of highly conserved pattern recognition receptors, in the differentiation of NSCs. We observed that NSCs expressed TLR2, TLR3 and TLR4, which were differentially regulated during NSCs differentiation. The activation of TLR4 inhibited NSCs differentiation to neurons. Investigation of the mechanisms showed that TNF- α plays a crucial role in LPS-mediated inhibition of NSCs differentiation to neurons. The knockdown of IRF3 attenuated TNF- α induction by LPS, restoring NSCs differentiation. These data indicate that TLR4 activation has a negative impact on neurogenesis. Supported by NIDA, DA012815, DA027550 and DA022177.

HIV-1 Nef Expression in Rat Hippocampus Induces Inflammatory Cytokines in Serum and Peyer's Patches in the GI Tract. *R Loucil*¹, G Chompre¹, M Cruz², C Appleyard², R Noel¹; ¹Biochemistry Department, Ponce School of Medicine, Ponce, PR 00731, ²Physiology Department, Ponce School of Medicine, Ponce, PR 00731.

Gastrointestinal pathology is a recurrent problem during HIV infection and disease progression. HIV enteropathy can often be associated with a specific cause, such as an opportunistic pathogen; however some HIV-positive individuals present idiopathic enteropathy that appears to involve inflammatory processes. In HIV associated dementia, the presence of increased LPS in plasma due to gastrointestinal inflammation suggests a brain-gut connection. Our previous work has shown that HIV-1 Nef expression by astrocytes in rat hippocampus produces spatial memory deficits, but did not address the possibility for systemic effects. Here we tested our rat model for inflammatory responses in the small intestine and blood. Primary rat astrocytes expressing Nef from a plasmid were unilaterally infused into the hippocampus of 30 day old Sprague Dawley rats. After a week, rats were euthanized and examined for systemic inflammation by serum cytokine analysis and macroscopic inspection of the small intestine for Peyer's patches. An increased number of Peyer's patches were found in Nef-treated animals that correlated with memory impairment. Both Nef and GFP control groups showed detectable RANTES in serum; however Nef animals uniquely showed increases in IL-1 β , IL-2, and TNF- α . These studies suggest that communication from brain to gut can be induced by known HIV-1 neurotoxins such as Nef. Supported by GM082406, RR003050.

Mechanism Underlying Methamphetamine Induced Autophagy and Apoptotic Death in Endothelial Cells. *J Ma*¹, S Ramakrishnan², H Yu¹, R Charboneau³, R Barke³, S Roy¹; ¹Department of Surgery, University of Minnesota, Minneapolis, MN 55455, ²Department of Pharmacology, University of Minnesota, Minneapolis, MN 55455, ³Department of Surgery, Veterans Affairs Medical Center, Minneapolis, MN 55417.

Methamphetamine (METH), a potent stimulant with strong euphoric properties, has a high abuse liability and is capable of dysregulating blood-brain barrier (BBB) function. Our previous studies have shown low dose (1 μ M) of METH treatment evokes an autophagic response in endothelial cells that is specific and initiated even in the absence of nutritional stress. Here we report that endothelial cells exposed to METH up-regulated Beclin -1 levels through kappa opioid receptor. METH ligation of kappa opioid receptor lead to inhibition of the mTOR signaling pathway by activation of Akt and Extracellular Signal-Related Kinase (ERK)1/2 signaling and antagonized by PD98059. Furthermore, progressively decreasing amounts of the apoptosis-inhibiting protein Bcl-2 were also found. Prolonged exposure to METH ultimately led to apoptosis via caspase8 dependent pathway but independent of caspase9 activation in endothelial cells. There was an increased number of early apoptotic cells (annexin V positive) following chronic METH exposure. Studies are in progress to determine if knock down of Beclin 1 levels by RNA interference decreases METH induced autophagy but accelerates METH-induced apoptosis. These observations provide a basis for interfering with the autophagic survival response following endothelial injury induced by chronic METH exposure. Supported by RO1 DA 12104 ; RO1 DA 022935; KO2 DA 015349, and P50 DA11806 (to S.R.)

Dynamics of Dendritic Cells and T Cells In HTLV-1-Associated Neuroinflammatory Disease: Implications in Immunomodulatory Therapies and Diagnostic Tools. *SL Manuel*¹, G Makedonas², MR Betts², J Gardner², JJ Goedert³, ZK Khan¹, B Wigdahl⁴, P Jain¹; ¹Drexel Institute for Biotechnology & Virology Research, Drexel University College of Medicine, Doylestown, PA 18902, ²Department of Microbiology and Immunology, University of Pennsylvania School of Medicine, Philadelphia, PA 19104, ³Division of Cancer Epidemiology and Genetics, National Cancer Institute, Bethesda, MD 20892, ⁴Institute for Molecular Medicine & Infectious Disease, Drexel University College of Medicine,

Philadelphia, PA 19102.

HTLV-1-associated myelopathy/tropical spastic paraparesis (HAM/TSP), is a debilitating neurodegenerative disease characterized by a robust immune response including the oligoclonal expansion of cytotoxic T lymphocytes (CTLs) specific for the viral oncoprotein Tax. However, the underlying mechanism resulting in the disease process is currently unknown. The CTL response is affected by many factors including the efficiency of epitope processing and presentation. In this respect, dendritic cells (DCs), the most potent antigen presenting cells, have long been recognized as key regulators of the immune system. We have previously demonstrated that DCs are capable of priming a pronounced Tax-specific CTL response in naive PBLs and in HLA-A*0201 transgenic mice. Since DCs are such crucial cells of the immune system, an extensive assessment of their function and interaction with T cells in HAM/TSP is critical. Therefore, utilizing a newly standardized DC and a pre-standardized T cell polychromatic antibody cocktail, we have investigated the immune activation of these cells in HTLV-1 infected samples from the Jamaican region including seronegative controls, asymptomatic carriers (ACs), and HAM/TSP patients. The extensive immune cell profiling was compared to the matched proviral loads and Tax mRNA levels leading to the identification of unique signatures distinguishing ACs from HAM/TSP patients. Collectively, these studies possess great potential to enable immune cell monitoring and development of diagnostic and therapeutic strategies for the HTLV-associated neuroinflammatory disease. Supported by NIH grant R01 AI077414 (NIAID) to Pooja Jain and a Philadelphia NeuroAIDS Research Training Grant T32 MH079785 (NIMH).

Bryostatin Protects Neurons Against HIV-Induced Inflammation. *R Mehla*¹, *S Bivalkar-Mehla*¹, *A Chauhan*¹; ¹Department of Pathology, Microbiology and Immunology, University of South Carolina, School of Medicine, Columbia, SC 29209.

Patients with prolonged HIV infection develop HIV associated neurocognitive deficits (HAND). Neurological complications during HIV infection are largely a result of direct neuronal damage imparted by either HIV-infected or uninfected macrophages and microglia. In the current study, we have demonstrated that among the proinflammatory cytokines, CXCL10 was distinctly upregulated over 1000 fold in activated astrocytes and macrophages. Consistently, cytokine profile from HIV-infected demented patient's CSF showed elevated levels of CXCL10 but not the HIV-infected non-demented patient's. Further, CXCL10 in combination with HIV synergistically enhanced neuronal toxicity and also revealed chemotactic activity (~ 40 fold) for PBMCs. These results suggest that signaling events induced by HIV and CXCL10 after binding to their respective surface receptors, CXCR4 and CXCR3 present on neurons must be intersecting. In accordance, blocking of CXCR3 and its downstream MAP kinases using specific inhibitor SB203580, showed suppression of neurotoxicity. Interestingly, bryostatin, a PKC modulator known to downregulate CXCR4 receptor expression in lymphocytes, showed strong suppression of CXCR4 in neuro-glial cells and conferred neuroprotection against HIV and CXCL10. In addition, bryostatin also suppressed HIV and CXCL10 induced PBMC chemotaxis and may be a potential neuroprotective agent. In conclusion, we have demonstrated the potential of bryostatin in neuroprotection in the setting of HIV infection. Supported by NIH.

Morphine Induces Spontaneous Sepsis in Mice by Modulating TLR Signaling. *JJ Meng*¹, *J Wang*², *J Ma*², *R Charboneau*³, *S Roy*¹; ¹Department of Pharmacology, University of Minnesota, Minneapolis, MN 55455, ²Department of Surgery, University of Minnesota, Minneapolis, MN 55455, ³Department of Surgery, Veterans Affairs Medical Center, Minneapolis, MN 55417.

Morphine is widely used for chronic pain management. Increased clinical reports showed that morphine may induce a group of adverse gastrointestinal symptoms such as constipation, bloating and nausea. In addition, a large amount of studies have been conducted to show that morphine treatment can suppress normal function of the intestinal immune system and contribute to induction of sepsis. However, the correlation between how morphine impairs gastrointestinal immune system and pathogenesis of sepsis is still illusive. In this study, we showed that morphine treatment induced extraintestinal growth of bacteria in blood, liver and mesenteric lymph node (mLN) in mice and the most frequently detected bacterial species were all commensal intestinal bacteria. Results from confocal microscopy revealed that morphine treatment disrupted the organization of tight junction proteins in small intestinal epithelium but did not show any effect on colon epithelium. Disruption of epithelial barrier lead to increased microbial translocation to the mLN and activation of mLN T cells to produce IL-17. Meanwhile, morphine treatment upregulated TLR2 and TLR4 expression in gut epithelial cells. Upregulation of TLR signaling in gut

epithelium induced production of IL-6, a proinflammatory cytokine which can regulate tight junction protein organization. Furthermore, the expression levels of miR155 and miR146a in gut epithelial cells were also modulated by morphine treatment, which suggested that the effects of morphine on TLR signaling are mediated by modulation of miR155 and miR146a expression. Supported by RO1 DA12104; RO1 DA022935; KO2 DA015349, and P50DA11806.

HIV-1 Transgenic Rats: Disruption of Sensorimotor Gating by Methamphetamine Challenge. *LM Moran*¹, *KM Webb*¹, *LL Hord*¹, *RM Booze*¹, *CF Mactutus*¹; ¹Behavioral Neuroscience Program, University of South Carolina, Columbia, SC 29223.

Since the introduction of combination antiretroviral therapy (cART) in the mid-90s, the most severe forms of HIV-1 induced cognitive deficits have diminished, however, HIV-1 associated neurocognitive disorders (HAND) remain as prevalent as before cART. Basic and clinical studies implicate alterations in the dopaminergic (DA) system in HIV-1 infection. Presently, we used the Fisher-344 HIV-1 transgenic (HIV-1 Tg) rat, which expresses 7 of the 9 HIV-1 genes, to examine potential DA alterations in the preattentive process of sensorimotor gating. HIV-1 Tg rats and non-transgenic controls (ns=8) were studied beginning at 35 days of age. At 48 hr intervals, animals were administered a single dose of methamphetamine (METH; 0, 0.5, 1, 2.5 and 5 mg/kg/ml SC) and individually tested for prepulse inhibition (PPI). Each animal was assessed using an acoustic startling stimulus [100dB(A) broad-band noise stimulus] and an acoustic prepulse [85 dB(A) broad-band noise stimulus] in a sound attenuating double-wall chamber with a continuous white noise 70dB(A) background. The protocol used a 5-min acclimation period, 6 startle trials, and 36 PPI trials [ISIs of 0, 8, 40, 80, 120, and 4000 ms, 6-trial blocks, Latin square design]. As the dose of METH increased, PPI of the startle response decreased, however, the HIV-1 Tg rats were significantly more responsive to the METH-induced disruption of PPI. Early-detected cognitive alterations in the preattentive process of sensorimotor gating may have significant predictive utility regarding the progression of DA alterations in HIV-1 infection. Supported by NIH grants DA013137 and HD043680.

Cannabinoid Receptor Expression on Peripheral Blood Mononuclear Cells of HIV Subjects and Marijuana Users by Flow Cytometry. *SM Munsaka*¹, *U Feger*¹, *M Andres*¹, *L Chang*¹; ¹Department of Medicine, John A. Burns School of Medicine, Honolulu, HI 96813.

Cannabinoids are known to have immunomodulatory effects, which are thought to be mediated primarily by CB2 receptors that are expressed on immune cells. However, immune cells also have CB1 mRNA and may express these receptors when the cells are activated. We developed a flow cytometry assay using anti-CB1-allophycocyanin and anti-CB2-biotin probed with streptavidin-phycoerythrin and measured CB1, CB2, activation markers and CCR5 expressions on lymphocytes (CD4+Th-cells, CD8+Tc cells, CD19+B cells) and on CD14+monocytes of seronegative (SN) controls (n=8), marijuana smokers (n=7), HIV+marijuana smokers (n=7), and HIV+ non-marijuana smokers (n=7). CD4+ cells of HIV+ marijuana users had lower CB1 expression (p=0.027) compared to those found in SN controls. Interestingly, CD8+ cells of marijuana smokers had higher CB2 expression (p=0.026) than in SN controls. Regardless of serostatus, marijuana users exhibited higher levels of inflammatory activated monocytes (CD14+CD16+) compared to non-users (p=0.002). Although there was no difference in the expression of the HIV chemokine co-receptor CCR5 between HIV+ subjects and SN controls (p=0.48), CCR5 expression tended to be higher among marijuana smokers than among non-users (p=0.11). These preliminary data suggest that HIV infection and marijuana-use affected CB1 and CB2 expressions and that smoking marijuana may increase monocyte activation and HIV chemokine co-receptor CCR5 expression, thus making these individuals more vulnerable to HIV infection. Supported by NIMH (2R01-MH61427); NIDA (2K24-DA16170); NINDS & NIDA (U54-NS56883).

Memory T-Cells Persisting in the Brain Following MCMV Infection Induce Long-Term Microglial Activation via IFN- γ Production. *MB Mutnal*¹, *S Hu*¹, *MR Little*¹, *JR Lokensgard*¹; ¹CIDMTR, University of Minnesota, Minneapolis, MN 55455.

Murine cytomegalovirus (MCMV) brain infection stimulates microglial cell-driven proinflammatory chemokine production which precedes the presence of brain-infiltrating systemic immune cells. Here, we show that in response to MCMV brain infection, antigen-specific CD8(+) T-cells migrated into the brain and persisted as long-lived memory cells. The role of these persistent T-cells in the brain is unclear because most of our understanding of antimicrobial T-cell responses comes from analyses of lymphoid

tissue. Strikingly, memory T-cells isolated from the brain exhibited an effector phenotype and produced IFN- γ upon restimulation with viral peptide. Furthermore, we observed time-dependent and long-term activation of resident microglia, indicated by MHC class II up-regulation and TNF- α production. The immune response in this immunologically restricted site, persisted in the absence of active viral replication. We then investigated the role of IFN- γ in chronic microglial activation by using IFN- γ -knockout (GKO) mice. At 30 d p.i., GKO mice demonstrated a similar phenotypic brain infiltrate when compared to wild-type mice (Wt), however, MHC class II expression on microglia isolated from these GKO mice was significantly lower compared to Wt animals. When IFN- γ producing CD8(+) T-cells were reconstituted in GKO mice, MHC class II up-regulation on microglial cells was restored. These results suggest that MCMV brain infection results in long-term persistence of antigen specific CD8(+) T-cells which produce IFN- γ and drive chronic microglial cell activation. Supported by NINDS R01 NS-038836.

Human Immunodeficiency Virus Type 1 (HIV-1) Infects Human Brain Pericytes In Vitro. S

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Human immunodeficiency virus type 1 (HIV-1) infection of the central nervous system (CNS) causes disruption of the blood-brain barrier (BBB) and contributes to the development of neurological dysfunctions. The BBB strictly regulates the transport of blood-borne cells and substances into the brain. Brain capillary endothelial cells are a major component of the BBB and have a dynamic interaction with other neighboring cells, namely, astroglia, pericytes, perivascular microglia, and neurons. The cross-talk between cells of the neurovascular unit is crucial for the formation and maintenance of a functional BBB. There are many reports related to the effects of HIV-1 on astrocytes, microglia, neurons, and endothelial cells. However, no study addressed the effects of HIV-1 infection on pericytes. The purpose of this study was to evaluate whether pericytes can be infected with HIV-1. To determine the expression of HIV-1 receptors on human brain pericytes, confocal microscopy and western blots were performed. Human brain pericytes expressed the HIV-1 receptor and co-receptors (CD4, CXCR4, and CCR5). Confirmation of HIV replication in human brain pericytes was performed by HIV-1 p24 ELISA. Low viral replication was detected after exposure to the X4 and R5 strains of HIV. These findings indicate that human brain pericytes can be infected with HIV-1. Infected pericytes may be involved in progression of the HIV-1-induced CNS damage. Supported by in part by ES 07380, DA027569, MH63022, MH072567, and NS39254.

Evidence of Neuroprotection by PDIA3 in SIV/Methamphetamine Rhesus Macaques. C Ninemire¹, G Pendyala¹, HS Fox¹; ¹Pharmacology and Experimental Neuroscience, University Nebraska Medical Center, Omaha, NE 68198.

Methamphetamine (METH) is a highly abused drug, and its use is particularly prevalent among HIV-infected individuals. The progressive state of neurodegeneration with the combination of HIV+METH is much more harmful than either one alone. The effect of METH on the brains of simian immunodeficiency virus (SIV)-infected rhesus macaques is an outstanding model for the drug abuse/HIV-infection scenario. We hypothesized that reactive changes would manifest by alterations in gene expression, and focused on the caudate nucleus. Microarray studies of the caudate revealed a significant increase for expression of protein disulfide isomerase family member A3 (PDIA3), known to be induced by cellular stress. qRT-PCR confirmed this increase in the caudate as well as the hippocampus of METH-treated SIV animals compared to SIV alone. PDIA3 has previously been implicated as a neuroprotective factor against prion neurotoxicity, therefore we hypothesized that over expression of PDIA3 is associated with a neuroprotective role in chronic SIV and METH comorbidity. In vitro studies using rat striatal neurons indicated a METH-concentration-dependent up-regulation of PDIA3 by confocal imaging. With PDIA3 responding to METH toxicity, could an increase in PDIA3 expression affect cell viability during METH treatment? In-vitro studies using SK-N-BE(2) neuroblastoma cells and siRNA for PDIA3 following METH treatments, led to a significant enhancement of cell toxicity. Conversely, over expression of PDIA3 reversed the effect implicating PDIA3 as a neuroprotective factor during METH neurotoxicity. Supported by NIDA, DA024467.

Impact of Chronic Opioid Exposure on HIV-1 Infection in the Bone Marrow. N. Parikh¹, B. Wigdahl¹, M. Nonnemacher¹; ¹Microbiology and Immunology, Drexel University College of Medicine, Philadelphia, PA 19102.

Intravenous drug use (IVDU) has been responsible for more than 36% of AIDS cases. Opiates are

known to modulate the immune system, augment susceptibility to HIV infection by increasing the number of chemokine receptors present on the cell surface and promote HIV disease progression by increasing viral replication. However, the molecular basis for accelerated HIV-associated disease progression and effects on cell physiology have yet to be fully understood. Within the bone marrow (BM) microenvironment, progenitor cells proliferate and give rise to precursor cells, which normally do not enter the peripheral circulation, however, upon changes in the chemokine levels, cells are induced to traffic into circulation. Stromal cell-derived factor-1 (SDF-1) is the major chemoattractant for hematopoietic progenitor cells (HPCs) and a CXCR4 ligand. Thus, opiate exposure boosts the number of chemokine receptors on the cell surface and increases levels of SDF-1, leading to increased trafficking of HIV-infected HPCs. We have established the CD34+ BM progenitor cell line, TF-1, as a model for HPC differentiation. The TF-1 cells express the HIV-1 receptor, CD4, and co-receptors, CXCR4 and CCR5 and thus, are susceptible to HIV-1 infection. We hypothesize that chronic opiate exposure impacts HIV-1-infected cell differentiation, proliferation, and/or cell viability and leads to modulation of chemokine levels allowing for trafficking of progenitor cells from the bone marrow compartment into peripheral circulation, thus accelerating HIV-1 disease progression. Supported by NIH/NIDA DC19807 and NIH/NINDS NS32092.

Methamphetamine Translocates Occludin to Endosomes in Human Cerebral Endothelial Cells. *M Park*¹, B Hennig², M Toborek¹; ¹Department of Neurosurgery, University of Kentucky Medical Center, Lexington, KY 40536, ²College of Agriculture, University of Kentucky, Lexington, KY 40536.

Methamphetamine (METH) is a drug of abuse with neurotoxic effects including blood-brain barrier (BBB) disruption. Indeed, the loss of occludin expression occurs as early as 1h after 10 μ M METH treatment in the membrane fraction of HCMEC/D3 cells (human cerebral microvascular endothelial cell line). The present study focused on the role of caveolin-1 (cav-1) as a possible modulator of METH-induced occludin changes in HCMEC/D3 cells. Exposure to 10 μ M METH resulted in increased actin polymerization and localization of phospho-cav-1 at the ends of actin filaments. In addition, occludin co-immunoprecipitation with cav-1 and actin was decreased in HCMEC/D3 cells treated with METH for 1 h. Exposure to METH resulted in a shift of occludin localization from caveolae fraction to endosome-rich fraction as determined by sucrose-gradient fractionation. These changes were associated with co-staining of occludin with Rab11, a late endosome marker. Blocking of actin polymerization with latrunculin A protected against alterations of occludin levels in the cellular membranes. The present findings suggest that under normal conditions occludin is localized in cav-1-rich regions; however, its localization is altered to the endosomal pathway upon METH exposure. The change of cav-1 dynamics by actin polymerization may be responsible for this phenomenon. The results of the present study suggest the role of cav-1 in the regulation of occludin localization to tight junctions. Supported by in part DA027569, MH63022, MH072567, and NS39254.

Suppression of Tat-Mediated Neurotoxicity and Glial Inflammatory Signaling through Modulation of the CC Chemokine Receptor 5. *EP Podhaizer*¹, PE Knapp², KF Hauser¹; ¹Department of Pharmacology & Toxicology, Virginia Commonwealth University, Richmond, VA 23298, ²Department of Anatomy & Neurobiology, Virginia Commonwealth University, Richmond, VA 23298.

CCR5 is critical to HIV-1 infection as well as aberrant HIV-1 gp120-mediated signaling. Additionally, CCR5 has been shown to interact on multiple levels with the mu opioid receptor, whose signaling in response to opiates, such as morphine, exacerbates HIV-1 Tat mediated inflammatory signaling and neurotoxicity. The Tat inflammatory signaling cascade involves a drastic increase in RANTES/CCL5, an endogenous ligand of CCR5, from glia. Therefore, we hypothesized that inhibition of CCR5 would interfere with Tat-mediated inflammatory signaling and suppress neuronal toxicity while potentially altering morphine-mediated exacerbation of these effects. To address this hypothesis, neurotoxicity in response to morphine (500 nM), Tat (100 nM), or the combination was assessed alone or following pretreatment with the CCR5 antagonist, Maraviroc (50 nM), in mixed neuron-glia cultures using time-lapse microscopy. CCR5 inhibition was neuroprotective towards treatments of Tat and Tat + morphine. To determine the mechanism(s) of this effect, nuclear translocation of the NF- κ B subunit p65, which is critical to glial mediated cytokine and chemokine release, was assessed using immunocytochemistry. Maraviroc blunted Tat mediated nuclear immunopositivity of p65 at times when RANTES has been shown to be upregulated by Tat (6 and 12 h), and suggests one mechanism by which CCR5 inhibition provides neuroprotection against Tat toxicity. Ongoing studies aim to extend these findings and identify the

contribution of both glial and neuronal CCR5 to the neurotoxic effects of Tat and morphine. Supported by NIH P01 DA019398 and T32 DA007027.

Physical Activity Protects Against Methamphetamine-Induced Blood-Brain Barrier Dysfunction. CS Rashid¹, MJ Seelbach¹, L Chen¹, IE András¹, B Hennig¹, KA Esser¹, M Toborek¹; ¹Graduate Center for Nutritional Sciences, University of Kentucky, Lexington, KY 40536.

Moderate voluntary exercise promotes overall health and decreases the risk of developing cardiovascular and cerebrovascular diseases. It is our hypothesis that exercise increases antioxidant potential of brain microvessels thereby attenuating CNS toxicity associated with drug abuse. To address this hypothesis, mice were subjected to voluntary wheel running for at least 5 weeks while sedentary mice had no access to running wheels. At the end of the exercise regimen, mice were injected with a single dose of methamphetamine (meth, 10 mg/kg) for 1 or 24 hrs. Exercise protected against meth-induced BBB dysfunction as evidenced by in situ brain perfusion. The brains of sedentary meth-treated mice showed elevated accumulation of sodium fluorescein compared to exercised and vehicle injected control mice, with the hippocampus appearing most susceptible. Consistent with our functional results, isolated brain microvessels from sedentary but not exercised mice showed meth-mediated disruption of tight junction proteins (claudin-5, occludin, and ZO-1) localization and contiguity. In addition, meth exposure induced oxidative stress in sedentary mice which was not seen in our exercised animals. Interestingly, exercise tended to attenuate meth-induced hyperthermia, which may also modulate BBB function. Taken together, these results indicate that exercise is a modifiable behavioral factor that protects against meth-induced CNS toxicity. Supported by 2T32DK7778-11, DA027569, MH63022, MH072567, NS39254, and from the University of Kentucky Center for Muscle Biology.

Down Regulation of Longevity Gene P66ShcA Rescues HIV-1- and Opiate-Induced T Cell Apoptosis. S Rehman¹, D Kumar¹, A Malhotra¹, M Husain¹, PC Singhal¹; ¹Department of Nephrology, North Shore-LIJ, 100 Community Drive, Great Neck, NY 11021.

Patients with HIV-1 infection and opiate addicts have been reported to develop loss of T cells. Both HIV-1 and opiates are known to induce cellular injury through the induction of oxidative stress. Recently, a pivotal role of p66ShcA protein has been identified in the generation of oxidative stress and induction of T cell apoptosis. We hypothesized that both HIV-1 and opiates stimulate the p66ShcA pathway which deactivates redox-sensitive stress response program (RSSRP). In the present study, we examined the effect of HIV-1 and opiates on the activation of the RSSRP in T cells both in vitro and in vivo. Both HIV-1 and opiates promoted ROS generation which was associated with T cell apoptosis. These effects of HIV-1 and opiates were attenuated in p66ShcA-knockout T cells as well as cells pre-treated by antioxidants such as SOD and catalase. Moreover, both opiates and HIV-1 enhanced T cell expression of phospho-p66ShcA and phospho-Foxo3A. In in vivo studies, both HIV-1 transgenic mice and mice receiving morphine not only showed enhanced splenic tissue ROS generation, apoptosis, expression of phospho-p66ShcA and phospho-Foxo3A but also displayed diminished production of antioxidants such as SOD and catalase. These findings indicate that both HIV-1 and opiates stimulate the p66ShcA pathway which deactivates RSSRP, resulting into the accumulation of ROS (by attenuating antioxidant generation) and thus facilitates the entry of T cells into apoptotic phenotype. Supported by National Institute of Health.

Effect of Opiates in a HIV-Infected Human Glia-Neuron Crosstalk System. M Rodriguez-Martinez¹, K Hauser², E Rios-Olivares¹, JW Rodriguez¹; ¹Department of Microbiology and Immunology, Universidad Central del Caribe School of Medicine, Bayamon, PR 00960, ²Department of Pharmacology and Toxicology, Virginia Commonwealth University, Richmond, VA 23298.

Since there is frequent use of opiates in the HIV-1 population, it is significant to appreciate how these drugs impact HIV-1 replication at the cellular and molecular level. Previous studies have indicated that methadone (MTD) enhances HIV-1 replication in microglia raising a major concern that MTD and perhaps buprenorphine (BUP) could also play a role in the pathogenesis of HIV-associated neurocognitive disorders (HAND). HIV-1 infection provokes immune activation of macrophages enhancing migration to the brain and secretion of neurotoxins affecting glial cells and neurons. In this study, we used an HIV-1 infected glia-neuron crosstalk system to investigate whether BUP and MTD modulate HIV-1 replication using quantitative RT-PCR, measuring pro-inflammatory cytokine production using a cytometric bead array (CBA), and neurotoxicity using MTT and caspase-3 assays. We show that both MTD and BUP enhanced HIV-1 replication in human glial cells in single culture and co-culture. This enhancement was

associated with a significant increase in the production of inflammatory cytokines. Furthermore, supernatants from single culture and co-culture of HIV-infected human glial cells treated with MTD and BUP augmented the neurotoxicity. These results suggest that addition of either MTD or BUP to an HIV-infected human glia-neuron system was detrimental for neuronal survival. The findings also provide further information about the cellular and molecular mechanisms through which MTD and BUP may modulate HAND pathogenesis. Supported by NIH-RCMI grant G12 RR03035.

Glycogen Synthase Kinase 3 β (GSK3 β) Inhibition Prevents Monocyte (Mo) Migration Across Blood Brain Barrier (BBB) via Rho/Rac Suppression. *S Rom*¹, *S Fan*¹, *H Dykstra*¹, *N Reichenbach*¹, *S Ramirez*¹, *Y Persidsky*¹; ¹Department of Pathology and Laboratory Medicine, Temple University School of Medicine, Philadelphia, PA 19140.

GSK3 β was identified as a potent regulator of immune responses. Our work showed GSK3 β inhibition in human brain microvascular cells (BMVEC) reduced adhesion molecules, monocyte adhesion and migration across BMVEC monolayers. Mo interactions with TNF- α activated BMVEC led to barrier disruption and GSK3 β suppression restored barrier integrity. Mo migration across BBB requires activation of small Rho GTPases regulating cytoskeleton. We hypothesize that GSK3 β inhibition in Mo blocks their adhesion/migration across BBB via GTPase inhibition. TNF- α BMVEC stimulation increased Mo adhesion/migration (3-4-fold) and Mo pretreatment with inhibitors resulted in decrease in adhesion (35-80%) and migration (50%). Using G-LISA assay measuring active, GTP-bound RhoA and Rac1, we demonstrated increase in GTP-RhoA (3.8-fold) and GTP-Rac1 (1.8-fold) in Mo stimulated with lysophosphatidic acid or LPS and significant inhibition by three inhibitors (19-63%). To mimic endothelial-cell Mo interactions we stimulated VLA4 by fibronectin or by cross-linking with CD49d antibody resulted in RhoA and Rac1 GTPases activation in monocytic cell line U937 (2 fold and 3 fold, respectively). Mo pretreatment with GSK3 β inhibitors significantly attenuated activation of both RhoA and Rac1 (50% and 35% decrease, respectively). Cells treated with GSK3 inhibitors showed increased levels of phosphorylated (inhibitory) sites of GTPase binding proteins, Cofilin and VASP indicating that inhibition of GSK3 directly affects cytoskeleton rearrangement and adhesion via suppression of Rho GTPases. Supported by AA015913 and MH65151.

Biodistribution and Efficacy of Nanoformulated Antiretroviral Drugs. *U Roy*¹, *P Dash*¹, *S Balkundi*¹, *PR Bathena*¹, *A Nowacek*¹, *L Poluektova*¹, *J McMillan*¹, *K Fletcher*¹, *Y Alnouti*¹, *H Gendelman*¹; ¹Department of Pharmacology and Experimental Neurosciences, University of Nebraska Medical Center, Omaha, NE 68198.

Antiretroviral therapy (ART) has substantively reduced mortality of HIV disease. However, complex dosing regimens and tissue toxicities limit therapeutic efficacy. To this end nanoformulated ART (nanoART) was developed to improve therapeutic outcomes. METHODS: Nanoparticle formulations of efavirenz (EFV), ritonavir (RTV) and atazanavir (ATV) were milled and characterized in cell-based laboratory assays (J. Contr. Rel. 2010). The best ART formulations were assessed for drug pharmacokinetics (PK), biodistribution and toxicity in normal Balb/C mice. PK and biodistribution of nanoART were determined by mass spectrometry following intraperitoneal, subcutaneous and intravenous administration. Drug efficacy was determined in combined immunodeficient mice reconstituted with human peripheral blood lymphocytes (hu-PBL-NOD/SCID) and infected with the HIV-1. RESULTS: PK studies of the nanoART combination in Balb/C mice revealed levels of all three drugs in serum, peaking at 6 hours (4 μ g/ml EFV, 2-3 μ g/ml ATV and 1.5-2 μ g/ml RTV) and detectable in liver, spleen, kidneys and lungs up to 14 days (0.1-10 ng/ml) post administration. Treatment of infected hu-PBL-NOD/SCID mice with combined nanoART resulted in a 100-fold reduction in viral antigen compared to control. CONCLUSIONS: After a single dose of nanoART, drug levels were detected in serum and tissues up to 14 days post-treatment and were effective in reducing viral levels in infected humanized mice. These studies demonstrate the potential of nanoART to simplify drug regimens and better elimination of HIV-1 from its tissue reservoirs. Supported by NIH.

Elevated Expression of HIV Viral Proteins in the Liver and Spleen of HIV-1 Transgenic Rats Treated with High Concentration of Ethanol. *S Sarkar*¹, *EF Langsdorf*¹, *C Liu*¹, *SL Chang*¹; ¹Institute of Neuro-Immune Pharmacology, Seton Hall University, South Orange, NJ 07079.

Ethanol concentration [alcohol by volume (ABV)] significantly differs in various alcoholic beverages ranging from 12% in wine to 40% or higher in hard liquors. Ethanol activates the hypothalamic-pituitary-

adrenal (HPA) axis, subsequently activating the supraoptic nucleus (SON). We have previously reported that SON was activated at 48%, 64%, or 80% ethanol, but not 16% or 32%. Therefore we hypothesized that choosing high ABV over low ABV beverages can result in different outcomes such as differential gene expression in health and disease. In this study, we have compared the gene expression of HIV viral proteins *tat* and *nef* in liver and spleen of HIV-1 transgenic rat model (rodent model of HIV patients on HAART). Animals were administered water, 20% or 52% ethanol (4.8 g/kg per-day) by gavage for 3 days. 2 hours after the final treatment blood was collected for serum preparation to determine corticosterone levels, liver and spleen were harvested for RNA isolation to determine expression of *tat* and *nef* using absolute quantitative real-time PCR (aqPCR) developed specifically for HIV viral proteins. No significant difference among groups was observed in the serum corticosterone levels. Our data shows that copy number of *tat* and *nef* is 52.35 ± 1.47 and 764.2 ± 165.4 in liver and 647.7 ± 91.85 and 22218 ± 1887 in spleen respectively. The *tat* and *nef* expressions are significantly higher in the animals given ethanol of 52% but not 20% in comparison to control. These results indicate that high ABV beverages can increase the expression of HIV-1 viral proteins in HIV infected patients on HAART. Supported by RC2 AA019415/K02 DA016149.

Effect of Antioxidant Response Modulation on HSV-Induced Oxidative Damage. *SJ Schachtele*¹, *S Hu*¹, *MR Little*¹, *JR Lokensgard*¹; ¹Center for Infectious Diseases and Microbiology Translational Research, University of Minnesota, Minneapolis, MN 55455.

Using a murine model of herpes simplex virus (HSV)-1 encephalitis, our laboratory has shown that microglial cells are the principle mediators of neuroinflammation during viral infection, including production of proinflammatory cytokines and chemokines. Addition of HSV-1 to purified murine microglial cell cultures results in the generation of reactive oxygen species (ROS), an additional mechanism to damage invading pathogens. ROS production, while beneficial in combating infection, can also cause irreparable harm through bystander damage to crucial host cells. Indeed, HSV-infected microglia induce neuronal oxidative damage and mediate neurotoxicity *in vitro*. Antioxidant responses, preferentially in astrocytes, have been shown to be neuroprotective during exposure to oxidative insults such as hydrogen peroxide. In the present study, we utilized the transducing power of lentiviral particles for the efficient delivery of the antioxidant transcription factor nuclear factor-E2-related factor 2 (Nrf2) and the antioxidant protein heme oxygenase-1 (HO-1) cDNA to murine astrocytes. Immunocytochemistry and western blot confirmed lentiviral delivery and functional expression of antioxidant protein. We hypothesize that this antioxidant protein overexpression in murine astrocytes will be protective from HSV-induced microglial cell-mediated neurotoxicity. Conversely, we hypothesize that delivery of short hairpin RNA for either Nrf2 or HO-1 to murine astrocytes will increase the neurotoxic effect of HSV-stimulated microglia. Supported by NIMH (MH-066703) and NIDA (T32DA007097).

HIV-1 Glycoprotein 120 Induces the Pro-Inflammatory Cytokine IL-6 via the NF- κ B Pathway and Methamphetamine can Synergistically Potentiate gp120-Mediated IL-6 Induction. *A Shah*¹, *A Kumar*¹; ¹Division of Pharmacology & Toxicology, University of Missouri - Kansas City, Kansas City, MO 64108.

HIV-1 gp120 facilitates the viral attachment and entry into the CNS leading to infection of various regions of the brain, resulting in HIV-1 Associated Neurocognitive Disorders (HAND). In this study, we sought to address whether gp120 can induce the pro-inflammatory cytokine interleukin-6 (IL-6). We also addressed whether exposure to methamphetamine can induce IL-6 expression in astrocytes, either alone or in synergy with gp120. The transfection of gp120 showed a time dependent induction of IL-6 mRNA and protein expression, with peak levels of 51.3 ± 2.1 fold and 11.6 ± 2.2 fold respectively. The induction of IL-6 could be successfully abrogated by gp120-specific siRNA. Using chemical antagonists and siRNA for the NF- κ B pathway, we demonstrated the involvement of this transcription factor in gp120-mediated IL-6 induction. Both I- κ B- α and IKK2 inhibitors could abrogate the gp120-mediated IL-6 induction by 56.5% and 60.8% respectively. In this study, we also report that exposure of SVGA cells to methamphetamine results in an increase in IL-6 expression by 4.9 ± 2.3 fold, which was blocked by mGluR5 inhibitor MPEP. The expression of IL-6 increased further (76.0 ± 12.1 fold) when meth exposure was combined with gp120 transfection. Together our results suggest NF- κ B to be a potential therapeutic target for HIV-1 infected patients. We also demonstrate that methamphetamine can act synergistically with gp120 to induce IL-6 expression. Thus, HIV-infected individuals who consume methamphetamine are at higher risk of developing neuro-inflammation than non-abusers.

Methamphetamine and HIV-1 Tat Cooperate to Down-Regulate a Prominent Pro-Survival Pathway in Astrocytes, the Wnt Signaling Pathway. A Sharma¹, X Hu², C Napier², L Al-Harhi¹; ¹Department of Immunology/ Microbiology, Rush University Medical Center, and Chicago CFAR, Chicago, IL 60612, ²Department of Pharmacology/Center for Compulsive Behavior & Addiction, Rush University Medical Center, and Chicago CFAR, Chicago, IL 60612.

Methamphetamine (Meth) exacerbates HIV-associated neurocognitive disorders (HAND). The mechanism by which Meth exacerbates HAND is not entirely clear but is likely to involve cooperation between Meth and HIV verotoxins such as the transactivator of transcription (Tat) to heighten dysregulation in the CNS. We evaluated the impact of Meth on the Wnt pathway in astrocytes transfected with Tat. Meth (10 μ M-1 mM) and Tat cooperated to downregulate Wnt/ β -catenin signaling by >35%, as measured by Topflash reporter activity. Meth and Tat also down-regulated LEF-1 transcript, a key downstream effector of the canonical Wnt pathway, by >30%. Meth also downregulated NFAT, an important mediator of non-canonical Wnt signaling by >70%. Interestingly, estrogen, an activator of β -catenin signaling, at physiological concentrations of 1.5 and 3 nM normalized individual Meth and Tat effects on Wnt signaling but not their combined effects. Collectively, these findings demonstrate that Tat and Meth cooperate to down-regulate Wnt signaling. The net outcome of which may be a critical mechanism for exacerbated Meth and Tat mediated pathogenesis in the CNS. Supported by NIDA 1R03 DA026723-01, Chicago D-CFAR P30 AI082151.

Phosphorylation of Serine Resides in both the First Intracellular Loop and C-Terminal Region Differentially Regulates the Signaling Properties of CCR5. C Song¹, L Zhang¹, TJ Rogers¹; ¹CILR, Temple University School of Medicine, Philadelphia, PA 19140.

The cross-talk between the Mu-opioid receptor and chemokine receptor CCR5 can play an important role in neuroimmune regulation. The heterologous desensitization of CCR5 by agonists of MOR, homologous desensitization by agonists of CCR5, is dependent on receptor phosphorylation. We hypothesized that phosphorylation of serine residues differentially regulates the biological functions of CCR5. Here we report that the phosphorylation of serine residues in the first intracellular loop, and the C-terminal region, differentially regulates signaling properties of CCR5. We examined the cell signaling activity of CCR5 in various serine phosphorylation states using HEK293 cells expressing either wild type or various serine mutants created by site-specific mutagenesis. For example, analysis of wild-type, or S63D and S63A, or S337A and S337D substitutions, shows that phosphorylation of serine 63 and S337 is important for both the calcium flux response and chemotactic activity. Moreover, experiments with S325A and S325D, S336A and S336D, S342A and S342D, indicate that the phosphorylation of these C terminal serines is critical for the chemotactic activity of CCR5, but not the calcium mobilization response. Taken together, our studies show that "regulation" of CCR5, by either homologous or heterologous desensitization, can be the result of phosphorylation of specific serine residues which potentially yields a receptor with selective partial functional activity. This "selective phosphorylation" mechanism adds a significant degree of precision to the regulation of this receptor. Supported by DA14230, DA16544, DA25532, and P30DA13429.

Exposure of TF-1 Hematopoietic Progenitor Cells to the Mu-Opioid Agonist DAMGO Leads to Altered Surface Expression of CXCR4 and Decreased HIV-1 Replication. M. Strazza¹, S. Passic¹, V. Pirrone¹, O. Meucci¹, B. Wigdahl¹, M. Nonnemacher¹; ¹Microbiology and Immunology, Drexel University College of Medicine, Philadelphia, PA 19102.

About one-third of the cases of human immunodeficiency virus type-1 (HIV-1) infection leading to acquired immunodeficiency syndrome (AIDS) in the United States have been attributed to injection drug use, frequently involving the abuse of opioids. Previous in vitro and in vivo studies addressing the role of mu-opioid agonists in altering levels of the co-receptor CXCR4, and consequent HIV-1 replication, have yielded contrasting results. The bone marrow (BM) is believed to be a potential anatomical sanctuary for HIV-1. In this study, the CD34+CD38+ human hematopoietic progenitor cell line TF-1 was used as a model to investigate the effects of the mu-opioid receptor-specific peptide DAMGO on CXCR4 expression as well as infection of undifferentiated hematopoietic progenitor cells. The results revealed the presence of the mu-opioid receptor-1 isoform (MOR-1) in TF-1 cells. Flow cytometry experiments indicated that treatment with DAMGO resulted in a shift in the relative proportion of CXCR4+ cells to the low-expressing phenotype. This result is correlated with a >3-fold reduction in replication of the CXCR4-utilizing HIV-1

strain IIIB, indicating a potential role for high level CXCR4 expression in sustaining infection within these cells. These experiments provide insight into the role of MOR-1-mediated signaling with respect to inhibition of viral replication in BM progenitor cells. Supported by NIH/NIDA: DC19807; NIH/NINDS: NS32092.

Culture Oxygen Affects Tat and Nef Toxicity in Rat Striatal Neurons. *LM Tiede*¹, EA Cook¹, B Morsey¹, H Fox¹; ¹Department of Pharmacology and Experimental Neuroscience, University of Nebraska Medical Center, Omaha, NE 68123.

Neurodegeneration associated with persistent human immunodeficiency virus infection continues to afflict infected individuals. Much of the research on its etiology focuses on viral protein neurotoxicity. In traditional tissue culture incubators, the oxygen level that cells experience is higher than in vivo conditions. These differences can alter experimental results, especially pertaining to mitochondria and oxidative metabolism. Using quantitative imaging techniques, we investigated the role that oxygen levels played in mitochondrial responses of rat striatal neurons to the HIV virotoxins Tat and Nef. We found that neurons cultured in physiological oxygen and atmospheric oxygen both exhibited significant increases in mitochondrial reactive oxygen species production. However, mitochondria of neurons cultured at physiological oxygen underwent depolarization while those cultured at atmospheric oxygen became hyperpolarized following treatment with Tat compared to controls treated with vehicle. Compared to controls treated with heat-inactivated Nef, native Nef treatment also increased mitochondrial reactive oxygen species production for both conditions, but resulted in mitochondrial depolarization and increased death only in neurons cultured in physiological oxygen. These results indicate a role for oxygen metabolism and redox balance in toxicity of viral proteins, and help elucidate mechanisms of neurotoxicity of HIV. Supported by National Institutes of Health grants MH073490 and MH062261, Nebraska Tobacco Settlement, NIAID Award 5 T32 AIO60547.

Speedball Enhances Cytokine Production in Presence of HIV Vpr. *L Torres*¹, LG Rivera¹, RJ Noel Jr¹; ¹Department of Biochemistry, Ponce School of Medicine, Ponce, PR 00716.

PURPOSE: Human Immunodeficiency Virus (HIV) infected individuals may be at an increased risk of developing neurological abnormalities. Viral proteins, such as Vpr, are considered to cause neuronal damage, which may be aggravated with concurrent drug use. Studies have examined the interactions between viral neurotoxins and individual drugs of abuse. However, no studies to date have examined the effect of multiple drug abuse, such as speedball, with HIV neurotoxins. **DESIGN METHODS:** Live rats were tested by the novel object and novel location tasks to assess cocaine and morphine effects in hippocampal-dependent learning and memory. Concurrent in vitro cell culture studies assessed the ability of speedball to alter Vpr-related induction of cytokines in astrocytes. **RESULTS:** Rats treated with cocaine have a significant increase ($p < 0.05$). Speedball enhances RANTES and IP10 in astrocytes expressing Vpr. **CONCLUSION:** Drug use is a prominent risk factor driving the HIV epidemic. Coincident drug abuse with HIV infection results in almost a doubling of the risk of neurocognitive disorders. We expect that speedball will aggravate Vpr neurotoxic effects by increasing in pro inflammatory and apoptotic factors resulting in exacerbated learning deficits. Supported by R03DA026722 and G12RR003050.

Astrocyte Elevated Gene-1 Contributes to HIV-1-Associated Dementia by Modulating Astrocyte Responses to Inflammation and Injury. *N Vartak*¹, K Borgmann¹, L Tang¹, A Ghorpade¹; ¹Department of Cell Biology and Anatomy, University of North Texas Health Science Center, Fort Worth, TX 76107.

A novel gene, astrocyte elevated gene-1 (AEG-1), was identified as an HIV-1 and tumor necrosis factor (TNF)-alpha inducible transcript in astrocytes. Subsequently, AEG-1 was found to be elevated in many cancers, and was identified as an oncogene responsible for metastasis, anchorage-independent growth and resistance to chemotherapeutic drugs. However, its role in HIV-1 infection has not been elucidated. Here, we propose that AEG-1 is a contributing factor to HAND/HAD progression, modulating astrocyte responses to inflammation, injury and stress by activating the downstream nuclear factor (NF)-kappa-B pathway. Our preliminary data in human astrocytes demonstrate that HAD-relevant stimuli upregulate the expression of AEG-1 at both mRNA and protein levels. A corresponding decrease in excitatory amino acid transporter (EAAT)-2 mRNA levels and an increase in CXCL8 chemokine production was also observed. This suggests a probable role of AEG-1 in HAD by modulating the expression of these proteins at the mRNA level. Further, our immunocytochemical studies show that AEG-1 localizes in the cytoplasm in response to HAD-relevant inflammatory stimuli but translocates to the

nucleolus in response to injury and oxidative stress. Time-lapse imaging studies were performed with AEG-1-GFP construct, to determine the intracellular localization of AEG-1 in astrocytes during migration and HAD-relevant stimuli-treatment. Differential intracellular AEG-1 localization in response to HAD-relevant stimuli and injury strongly implicates AEG-1 in regulating astrocyte behavior in HAD and astrogliosis. Thus, AEG-1 may link astrocyte injury responses to the development and progression of malignant astrocytomas and HAD. Supported by NINDS/2R01NS48837-06.

HIV-1 Envelope Evolution and Cognitive Dysfunction Progression in Puerto Rican Women Infected with HIV-1. *FJ Vázquez-Santiago*¹, LM Meléndez², M Plaud-Valentín², V Wojna², RJ Noel Jr¹, V Rivera-Amill¹; ¹Department of Microbiology, Ponce School of Medicine, Ponce, PR 00732-7004, ²Department of Microbiology and Medical Zoology, University of Puerto Rico Medical Sciences Campus, San Juan, PR 00936-5067.

HIV associated neurocognitive disorder (HAND) is a clinical neurodegenerative manifestation caused by HIV-1 neuroinvasion. Neurodegeneration has been related, among other factors, to both the direct and indirect actions of gp120 within the central nervous system (CNS). The main purpose of this project is to characterize the contribution of HIV envelope (env) evolution to neurological impairment by studying viral evolution and compartmentalization and correlating them with disease severity and cognitive status/decline in HIV-1-infected women with cognition impairments. We hypothesize that specific sequences changes within env are linked with progression to HIV-1 associated dementia (HAD). Viral RNA was extracted from plasma and cerebrospinal fluid collected from patients at various time points. Gp120, including C2V3 region, was amplified by PCR and the product cloned into pCR2.1, and sequenced. Sequencing data showed 4.8% divergence of plasma virus between samples obtained before and after neurological progression, with the later sample showing 40% greater diversity among all envelopes sequenced. In CSF, the divergence was lower than in plasma sequences but exhibited the same trend; the sequences obtained after progression showed greater divergence and diversity. Sequences in plasma and CSF reflected increased viral diversity at or near the time of progression, which suggests envelope evolution may be a contributing factor for development of neuropathology. Supported by NCRR-RCMI/ RR003050; R01-MH08316-01; and SNRP-NINDS-1-U54NS431.

Involvement of 4-Aminopyridine-Sensitive K⁺ Current in Methamphetamine-Induced Hippocampal Neuronal Apoptosis. *J Wang*¹, H Xiao², H Xiong¹; ¹Department of Pharmacology and Experimental Neuroscience, University of Nebraska Medical Center, Omaha, NE 68198-5880, ²Department of Toxicology, School of Public Health, Nanjing Medical University, Nanjing, China 210029.

Methamphetamine (Meth) is an illicit psychostimulant that is widely abused in the world. It is reported that Meth abuse leads to neuronal damage, such as loss of gray matter in the cortices and the shrinkage of hippocampi. However, the mechanism is still largely unknown. K⁺ homeostasis is believed to be closely related with cell volume, and persistent K⁺ efflux results in apoptotic volume decrease. In the present study, we explored whether K⁺ channels were involved in Meth-induced apoptosis in primary rat hippocampal neuronal cultures. Using state-of-the-art whole-cell patching techniques, we observed that bath application of Meth differentially increased the amplitude of 4-aminopyridine (4-AP)-sensitive K⁺ current, but not the K⁺ current sensitive to tetraethylammonium (TEA), which is in line with the toxicological results that the Meth-induced neuronal apoptosis was significantly blocked by 4-AP, but not TEA. Further investigation revealed that Meth enhanced 4-AP-sensitive K⁺ current by inhibition of ERK phosphorylation. These results, taken together, suggest that Meth increases 4-AP-sensitive K⁺ current by inhibition of ERK phosphorylation, leading to apoptotic volume decrease and ultimate neuronal apoptosis. Supported by NIH 2R56 NS041862-10A1.

Morphine Suppresses the Cellular Restriction Factors of AIDS Virus Infection. *YZ Wang*¹, X Wang¹, L Ye¹, JL Li¹, L Song¹, N Fulambarkar¹, WZ Ho¹; ¹Department of Pathology & Laboratory Medicine, Temple University School of Medicine, Philadelphia, PA 19140.

A key facet of innate defense mechanisms against retroviruses is the presence of intracellular restriction factors. Although opioids, such as morphine, enhance HIV/SIV infection and replication, the mechanism(s) of the action remains to be determined. Thus, we investigated the impact of morphine on cellular restriction factors of AIDS virus, HIV and SIV. We demonstrated that morphine treatment of human blood mononuclear phagocytes significantly inhibited the expression of several key elements (RIG-I, IRF-3, IRF-5 and APOBEC3C/3G) in type I IFN pathway. The suppression of these innate

restriction factors was associated with reduced production of type I IFNs and the enhancement of HIV or SIV infection of macrophages. These findings indicate opioid use impairs cellular innate anti-HIV/SIV mechanism(s) in macrophages, contributing to cell susceptibility to AIDS virus infection. Supported by National Institutes of Health grants DA12815, DA22177 and DA27550 (W-Z.H.).

Mycophenolate Mofetil Inhibits Hepatitis C Virus Replication in Hepatocytes. L Ye¹, JL Li¹, X Wang¹, YZ Wang¹, H Parekh¹, WZ Ho¹; ¹Department of Pathology and Laboratory Medicine, Temple University School of Medicine, Philadelphia, PA 19140.

Hepatitis C virus (HCV) infection is a leading indication for liver transplantation and a major cause of graft failure. Cyclosporine A (CsA), a widely used immunosuppressant for people who receive organ transplantation, has been recognized to have the ability to inhibit HCV replication both in vivo and in vitro. In this study, we investigated the effects of several other immunosuppressants, including mycophenolate mofetil (MMF), Rapamycin (Sirolimus) and FK506 (Tacrolimus), on HCV infection/replication in human hepatocytes. MMF treatment of hepatocytes before or during HCV infection significantly suppressed full cycle viral replication, as evidenced by decreased expression of HCV RNA, protein and production of infectious virus. In contrast, Rapamycin and FK506 had little effect on HCV replication. Investigation of the mechanism(s) disclosed that the MMF's inhibition of HCV replication is due to its depletion of guanosine, a purine nucleoside crucial for synthesis of guanosine triphosphate (GTP), which is required for HCV RNA replication. We found that the supplementation of cell cultures with exogenous guanosine could reduce MMF-mediated anti-HCV activity. These data indicate that although MMF is a potent immunosuppressant, it may be beneficial for people infected with HCV. Future studies are needed to determine clinical significance of the in vitro anti-HCV effect of MMF. Supported by NIDA DA12815, DA22177 and DA27550.

ZO-1 Nuclear Translocation by Rho Signaling is Involved in HIV Tat-Induced Alterations of Claudin-5 Expression. Y Zhong¹, SY Eum¹, B Hennig², M Toborek¹; ¹Department of Neurosurgery, University of Kentucky, Lexington, KY 40536, ²College of Agriculture, University of Kentucky, Lexington, KY 40536.

HIV Tat protein can contribute to the dysfunction of brain microvascular endothelial cells (BMEC) and facilitate HIV entry into the brain. Rho signaling plays an essential role in HIV-mediated disruption of the BBB integrity; however, the mechanisms involved in these effects remain unclear. In the present study, we hypothesize that ZO-1 serves as a scaffold for the assembly of Rho signaling molecules with the transmembrane tight junction proteins and that this association may be disrupted by Tat protein. Indeed, exposure to Tat activated the Rho signaling, increased ZO-1 translocation into the nuclei, and decreased claudin-5 levels. These effects were accompanied by increased binding activity of transcription factor CREB to the proximal region of the ZO-1-promoter. Inhibition of the Rho signaling cascade by the C3 exoenzyme, ROCK inhibitor Y27632 or MLC inhibitor ML-9 blocked Tat-induced CREB activation and ZO-1 nuclear translocation. In a series of experiments with CREB-depleted and ZO-1-depleted cells, we demonstrated that MLC phosphorylation was important for Tat-induced localization of ZO-1 into the nuclei. Interestingly, inhibition of nuclear translocation of ZO-1 protected both against Tat-mediated activation of CREB DNA binding and claudin-5 disruption. These findings suggest that the Rho signaling pathway can disrupt the integrity of tight junctions in response to Tat treatment by stimulating nuclear translocation of ZO-1, which then induces a decrease in claudin-5 expression. Supported by DA027569, MH63022, MH072567, and NS39254.

HIV-1 Tat and Morphine Affect Calcium Levels and Viability of Oligodendroglia: Evidence for NMDA Effects. S Zou¹, S Fitting², KF Hauser², PE Knapp¹; ¹Department of Anatomy and Neurobiology, Virginia Commonwealth University, Richmond, VA 23298, ²Department of Pharmacology and Toxicology, Virginia Commonwealth University, Richmond, VA 23298.

Myelin pallor is frequently reported in HIV patients, and can occur prior to other evidence of CNS disease. This has largely been attributed to edema resulting from blood brain barrier pathology, but we also found that HIV-1 Tat can directly affect oligodendroglia (OLs) in culture and that OLs in the CNS of Tat transgenic mice show pathology (Hauser et al, *Glia*, 2009). Studies were conducted to determine if direct effects of HIV-1 Tat on OL survival or other functional or phenotypic changes are mediated through interaction with NMDA receptors. We used a computer-assisted imaging system to track individual cells at 30 min intervals over 48 hr in vitro. Both immature OLs (morphology: multiple thin, branching processes)

and mature OLs (morphology: several thick processes and myelin-like membranes) treated with HIV-1 Tat had a dose-dependent (1-100 nM) decrease in survival. Initial studies in an infectious culture model showed similar toxicity. At both stages of maturation Tat induced a gradual increase in $[Ca^{2+}]_i$ that was attenuated by MK801 but not by CNQX. We also examined interactions between Tat and the mu-opioid agonist morphine. Morphine by itself elevated $[Ca^{2+}]_i$ in both immature and mature OLs in a naloxone-reversible manner. The kinetics and amplitude of $[Ca^{2+}]_i$ response to combined Tat and morphine exposure was variable between individual cells, pointing to heterogeneity even among OLs at a similar stage of differentiation. Our results provide evidence that OLs are targets of HIV/viral proteins and/or opiates, and suggest that glutamatergic mechanisms are involved. Supported by DA24461; DA19398; and NS69216.

POSTER SESSION II: GENERAL ABSTRACTS

(in alphabetical order by presenting author)

Synaptodendritic Alterations Induced by HIV-1 Tat in Hippocampal Neurons. *MV Aksenova*¹, *MY Aksenov*¹, *S Bertrand*¹, *CF Mactutus*¹, *RM Booze*¹; ¹Department of Psychology, University of South Carolina, Columbia, SC 29208.

Cognitive deficits in normal aging, non-viral neurological disorders, and HIV-associated neuropathology are reflective to the aberrant structure of synaptodendritic networks. Evidence of the morphological neurodegenerative triad of dendritic spine loss, dystrophic neurites, and dendritic simplification in the brain burdened by chronic HIV-1 infections suggests that advanced analysis of dendritic morphology and synaptic densities is essential for proper assessment of neuronal injury resulting from the exposure to neurotoxic viral proteins. The cytoskeleton of dendritic spines is primarily made of filamentous actin (F-actin), which makes the fluorescent phalloidin (specific F-actin-binding peptide probe) staining an excellent method for the analysis of the spine morphology. In this study we report experiments demonstrating that treatment of hippocampal cultures with 50 nM dose of Tat 1-86 B for 4-24 hours results in the decreased labeling of neuronal dendrites with fluorescent phalloidin. Results suggest that early events in the process of Tat neurotoxicity involve F-actin de-polymerization and/or degradation of the F-actin-rich synaptodendritic network. The actin cytoskeleton directly determines the morphology of the spine, which is known to rapidly change in responses to stimuli. Supported by University of South Carolina Research Foundation grant and NIH Grant DA013137.

Alcohol Redistributes Membrane Lipids and Calcium Permeable AMPA Receptors to Focal Dendrite Microdomains. *M Bae*¹, *H Xu*¹, *VR VV Ratnam Bandaru*¹, *NJ Haughey*¹; ¹Department of Neurology, Johns Hopkins University School of Medicine, Baltimore, MD 21287.

HIV-infected subjects who abuse alcohol exhibit deficits in cognitive tasks that assess frontal lobe function compared with HIV-infected subjects who do not abuse alcohol. The mechanisms how alcohol can accentuate the toxic effects of HIV products and viral proteins have not been identified. Here we suggest that alcohol impairs neuronal function by modifying the membrane lipid composition, resulting in AMPA receptor mislocalizations. Ethanol (0.03-0.3%) extracted neuronal membrane lipids within 2min, with the following efficiency: Cholesterol > ceramide > sphingomyelin. Lipids recovered within 12h, suggesting that new synthesis was required to restore the lipids. Based on evidence that lipid rafts where cholesterol/ceramide enriched are important for the surface expression of AMPA-type glutamate receptors, we determined if ethanol disrupted the AMPA receptor locations. Ethanol caused a collapse of ganglioside (GM1) enriched lipid rafts and increased the colocalization of GluR1 with GM1. Thus, ethanol disrupted membrane lipid compositions and redistributed AMPA receptors to create high receptor density areas. These observations were consistent with functional studies in which an ethanol exposure followed by AMPA applied during ethanol washout increased the amplitude of focal calcium bursts in AMPA receptor concentrated regions with large dendrite regions unresponsive to AMPA. Thus, alcohol abuse may contribute to neuronal dysfunction in HIV-infected subjects by perturbing plasma membrane structure and mislocalizing calcium permeable AMPA receptors. Supported by NIH grant R01AA017408.

The Effect of gp120 on the Antinociceptive and Neurophysiological Effects of Morphine. *X Chen*¹, *LG Kirby*¹, *J Palma*¹, *EB Geller*¹, *TK Eisenstein*¹, *MW Adler*¹; ¹Center for Substance Abuse Research,

Temple University School of Medicine, Philadelphia, PA 19140.

Recently we showed that morphine's analgesic activity can be attenuated by chemokines, specifically CCL5 and CXCL12. The HIV-1 coat protein, glycoprotein 120 (gp120), binds to the same respective receptors as do these chemokines. Hence, the present experiments were designed to investigate the effect of gp120 on morphine antinociception in rats. Morphine, with or without gp120, was infused into the PAG of young adult, male Sprague-Dawleys via a surgically implanted cannula, or given subcutaneously (sc), and antinociception assessed using the cold-water (-3°C) tail-flick test. It was found that (1) pretreatment with gp120 itself (10-133 ng, PAG) had no nociceptive effect; (2) gp120 (25 or 100 ng) dose-dependently reduced the antinociception induced by sc injected (3 or 6 mg/kg) or PAG administered (100 ng) morphine; (3) the inhibitory effect of gp120 on morphine-induced antinociception was reversed by AMD 3100, an antagonist at CXCR4. The effect of gp120 on mu-opioid receptor-mediated effects in PAG slices was also examined via whole-cell patch-clamp recordings. Pretreatment of slices with gp120 (200 pM) prevented morphine (10 uM)-induced hyperpolarization and reduction of input resistance in PAG neurons. Electrophysiology studies paralleled gp120-induced desensitization of a mu-opioid receptor-mediated response in PAG neurons at the single-cell level. These studies are the first to demonstrate that the analgesic activity of morphine can be reduced by the presence of gp120 in the PAG and that pretreatment with AMD 3100 is able to restore the analgesic effects of morphine. Supported by in part NIDA Grants DA 06650 (MWA) and DA 13429 (MWA) and DA 20126 (LGK).

Morphine-Induced Reversible Inhibition of Renal Tubular Secretion of ⁹⁹mTc-MAG3 In Mice. *K Cheng*¹, *K Bhagarva*², *D Kumar*¹, *D Salhan*¹, *A Malhotra*¹, *CJ Palestro*², *S Gupta*³, *PC Singhal*¹;
¹Feinstein Institute for Medical Research, Long Island Jewish Medical Center, New Hyde Park, NY 11040, ²Department of Nuclear Medicine, Long Island Jewish Medical Center, New Hyde Park, NY 11040, ³Liver Center, Albert Einstein College of Medicine, Bronx, NY 10461.

Purpose: Kidney plays a key role the excretion of opiate metabolites in general, and morphine in particular. In the present study, we evaluated the effect of morphine metabolite excretion on renal tubular secretion of ⁹⁹mTc-MAG3 in mice. Methods: Healthy FVB/N mice (n=3 each) were implanted with 75 mg morphine pellet s.c. for 72 hours. The mice were injected with 100 µCi of ⁹⁹mTc-MAG3 via the tail vein and imaged with a gamma camera. After image acquisition, the morphine pellet was removed. Mice were imaged again after 72 hours. Control mice were implanted with placebo. The acquisition protocol consisted of sixty-second images followed by twenty-nine minute images. Regions of interest were drawn around each kidney to generate time-activity curves. Split renal function & time to peak activity (Tpeak) were determined. Results: Split renal function was not significantly different between morphine-treated and placebo mice (P>0.05). The peak time of ⁹⁹mTc-MAG3 excretion in morphine-treated mice was significantly longer than placebo, 24.6±3.0 min vs 1.3±0.5 min. After 72 hours pellet removal, ⁹⁹mTc-MAG3 excretion was reversed to 2.0±1.7 min, and thus indicating restoration of tubular function. Conclusions: Morphine metabolites compete with other drugs for their tubular secretion. This effect, at least after short-term administration, appears to be reversible. Further examine of the mechanisms of tubular transport of ⁹⁹mTc-MAG3 and its interaction with morphine metabolites will provide a basis for understanding the tubular function abnormalities in opiate addicts.

Role of MEKK3 and TAK1 in C3 Gene Regulation by IL-1β. *P Datta*¹, *J Rappaport*¹; ¹Neuroscience, Center for Neurovirology, Temple University, Philadelphia, PA 19140.

Uncontrolled synthesis of the complement component C3 by HIV-1 induced proinflammatory cytokine IL-1β in the brain can lead to neuroinflammation and neurodegeneration. We have demonstrated earlier that IL-1β induces C3 synthesis in astrocytes and monocytes in a C/EBP dependent manner. We now investigated the role of MEKK3- and TAK1-dependent pathways in IL-1β mediated C3 gene regulation. Our studies demonstrate that overexpression of MEKK3 and TAK1 in astrocytes and monocytes induce C3 promoter activity. On the contrary, overexpression of kinase defective mutants of MEKK3 and TAK1 inhibited transcriptional response of the C3 promoter by IL-1β. These observations demonstrate that targeting MEKK3 and TAK1 may have potential as novel therapeutic strategies for inhibiting IL-1β mediated C3 gene regulation. Supported by NIH-NIDA.

Opioids Block the Effects of the HIV Entry Inhibitors Maraviroc and AMD-3100 in CNS Glia. *N El-Hage*¹, *SM Dever*¹, *T Ahmed*¹, *Y Zhang*², *KF Hauser*¹; ¹Department of Pharmacology & Toxicology, Virginia Commonwealth University, Richmond, VA 23298, ²Department of Medicinal Chemistry, Virginia

Commonwealth University, Richmond, VA 23298.

There are currently 27 HIV medications used in various combinations to treat HIV and AIDS, including inhibitors of viral entry. Maraviroc is a CCR5 inhibitor used with other HIV medications to treat CCR5 (R5)-tropic HIV and AMD-3100, a CXCR4 antagonist, is used to reduce CXCR4 (X4)-tropic HIV levels. Since these two inhibitors have the ability to inhibit HIV entry in target cells and opioid abusers are more susceptible to the neurodegenerative effects of HIV in the CNS, the goal of this study was to investigate to the impact of opioids such as morphine, widely abused drugs among people infected with HIV as well as DAMGO, on the inhibitory effects of Maraviroc and AMD-3100 on HIV entry in human microglia and astrocytes. We first confirmed that astrocytes and microglia express CCR5 and CXCR4 using flow cytometry. HIV binding and entry were directly visualized by confocal microscopy using GFP-labeled R5 (BaL) and X4 (NL4-3) virions and infection was further confirmed using a HIV Tat-activated luciferase reporter assay. As expected, we found that Maraviroc inhibited R5 HIV entry and reduced HIV infection levels by 95% in astrocytes and microglia. However, morphine and DAMGO treatment compromised the anti-HIV entry effects of Maraviroc leading to increased HIV levels in these cells. Similar results were found with opioids and AMD-3100 using X4 HIV. Our data suggest that opioids impair the effects of HIV entry inhibitors and may contribute to increased susceptibility of HIV entry in opioid abusers which could lead to accelerated CNS neuropathogenesis in these individuals. Supported by NIH P01 DA019398 R03DA026744.

Protein Phosphatase 2A Activation Leads to Threonine Dephosphorylation of Occludin in Human Brain Endothelial Cells Exposed to PCB153. SY Eum¹, IE Andras¹, JJ Choi¹, M Toborek¹; ¹Department of Neurosurgery, University of Kentucky, Lexington, KY 40536.

Occludin is an integral transmembrane protein associated with tight junctions, contributing to the physical gate function of the blood-brain barrier (BBB) of brain endothelial cells. Exposure of human brain endothelial cells to highly ortho-substituted non-coplanar polychlorinated biphenyls (PCBs) including PCB153, persistent environmental pollutants, results in the disruption of BBB which is accompanied by the decrease of tight junction proteins such as occludin and ZO-1. We hypothesized that the change of phosphorylation status of the protein can be involved in regulation mechanism of the occludin level in the cells. To assess the phosphorylation level, occludin was isolated using the immunoprecipitation method and Western Blotting was employed with anti-phosphorylated amino acid antibodies. Exposure of human brain endothelial cell line (hCMEC/D3) to PCB153 decreased phosphorylation level of threonine residues in 15min and return to basal level at 60min after treatment. In contrast, the other phosphorylated amino acid residues, tyrosine or serine, did not significantly changed by exposure to PCB153. Blockage of protein phosphatase 2A (PP2A) activity using specific inhibitors, Calyculin A, Cantharidine, and Fostriecin, prevented the threonine dephosphorylation and the decreased protein level of occludin in hCMEC/D3 cells exposed to PCB153. These results suggest that the PP2A-mediated threonine dephosphorylation is a critical step in PCB153-induced occludin disruption which can cause the dysfunction of tight junctions and BBB. Supported by American Heart Association 09SDG2300037, NIH/P42 ES 07380, MH63022, MH072567, NS39254.

Dopamine Modulation of Macrophage Function may Facilitate Development of HIV-Associated Neurological Disorders. PJ Gaskill¹, JW Berman¹; ¹Pathology, Albert Einstein College of Medicine, Bronx, NY 10461.

Macrophages are a key cell type in the development of HIV-associated neurological disease (HAND), not only as a primary target for HIV but also as the primary immune response to HIV infection in the CNS. The neurological complications of HIV infection are a major health issue among HIV-infected drug abusers. This could be due, in part, to increased CNS dopamine levels mediating the effects of drug abuse. We previously showed that dopamine increases HIV replication in human macrophages through activation of D2-like dopamine receptors (DR), increasing the number of HIV infected macrophages. Our current data indicate that activation of macrophage dopamine receptors also affects the functions of uninfected macrophages, potentially contributing to the dysregulated inflammatory response seen in NeuroAIDS. We show that dopamine modulates production of the inflammatory cytokines TNF- α , CCL2, IL-8 and IL-6 and alters the activation of macrophage signaling pathways. Our findings also demonstrate that macrophages express the dopamine transporter, and the dopamine synthesis proteins, tyrosine hydroxylase and DOPA-decarboxylase. We also show that in addition to DR1 and DR2, macrophages express DR3 and DR4. These data indicate that dopamine is important in the modulation of macrophage

function(s) and suggests a significant role for dopamine in the response to and development of HIV infection in the CNS. Further, these data suggest the drug-induced increases in CNS dopamine may be a common mechanism by which drugs of abuse exacerbate the development of HAND in HIV infected drug-abusers. Supported by NIDA.

Linkages Between Cannabinoid 2 Receptor Activation and CD38. *S Gorantla*¹, HE Gendelman¹, L. Poluektova¹; ¹Department of Pharmacology and Experimental Neuroscience, University of Nebraska Medical Center, Omaha, NE 68198.

Attenuation of neuroinflammation during HIV-1-associated brain disease remains an attractive means to restore neural homeostasis. One possibility is through Cannabinoid type 2 receptor (CB2R) agonists which have shown considerable therapeutic potential in their abilities to control neuroinflammation. Towards this end, a highly selective CB2R agonist, GP1a was seen to reduce leukocyte infiltration and microglial activation in a murine HIV-1 encephalitis (HIVE) model. We now show that GP1a down-regulates CD38, a multifunctional ecto-enzyme involved in calcium transport, expression on immunocytes. As a receptor CD38 participates in cell adhesion and a range of inflammatory responses that include immune activation. It is cited to reflect disease progression for HIV-1 infection and to mediate microglial activation by increasing intracellular calcium and nitric oxide production. CD38 may influence CB2 receptor mediated immunosuppression. Hence, we investigated the relationship between CB2R activation and regulation of CD38 expression. LPS activated human primary microglia and monocyte-derived macrophages (MDM) treated with CB2R agonists showed diminished CD38 expression by realtime RT-PCR and flow cytometry. Intracellular calcium levels and reactive oxygen species were also diminished following CB2R agonist treatment of microglia and MDM. We conclude that the selective activation of CB2 receptor can be a pharmacological approach to modulate CD38 expression during neuroinflammatory conditions including HIVE and neuroAIDS.

Mechanisms of BBB Damage and Hemorrhagic Stroke in Chronic Methamphetamine Abuse. *J Haorah*¹, MA Muneer¹, S Alikunju¹, A Szlachetka¹; ¹Department of Pharmacology and Experimental Neuroscience, University of Nebraska, Omaha, NE 68198.

We examine the association between blood-brain barrier leakage and hemorrhagic stroke observed in our animal model of chronic METH intake. We hypothesize that oxidative damage of VEGFR2 protein regulates VEGF signaling pathway for activation of MMP-2, -3, -9. To test the hypothesis, mice were given 15 mg METH/kg body weight daily by i.p. injection for 5-6 weeks. Permeability and immune cell infiltration across the BBB were assessed by Evan Blue and monocyte migration assays. Tight junction proteins, VEGFR-2, MMPs and TIMPs were analyzed by proteome assay and Western blot. ELISA kits assayed VEGF levels. Mechanisms of MMPs activation by VEGF via Src kinase signaling pathway was determined in brain endothelial cell culture with/without acetyl-L-carnitine (antioxidant) or PP2 (Src kinase inhibitor). After METH administration, we observed hemorrhagic injury, BBB damage and leakiness that were positively associated with MMPs activation and VEGFR-2 reduction. Decrease in VEGFR-2 correlated increase in VEGF levels, oxidative marker, Src phosphorylation and MMPs activation. Oxidative stress regulated this complex pathway because ALC and PP2 significantly mitigated the METH-induced BBB damage and hemorrhagic injury. Treatment of hBECs with exogenous VEGF-A was able to validate the role of VEGF for MMPs activation in relation to VEGFR-2 expression during METH-induced stress condition. These findings suggest that VEGFR-2 is extremely sensitive to oxidative stress, in which increase in intracellular VEGF levels activate MMPs in a p-Src-dependent manner leading to BBB loosening and hemorrhagic stroke. Supported by Faculty retention funds.

Morphine Potentiates Neuropathogenesis of SIV Infection in Rhesus Macaques. *R Hegde*¹, SM Bokhari¹, S Callen², H Yao², I Adany¹, Z Li¹, PD Cheney¹, O Narayan¹, S Buch²; ¹Department of Molecular & Integrative Physiology, University of Kansas Medical Center, Kansas, KS 66160, ²Department of Pharmacology & Experimental Neuroscience, University of Nebraska Medical Center, Omaha, NE 68198.

Despite the advent of antiretroviral therapy, HIV-1 infection and its complication with concurrent drug abuse is becoming an emerging problem. The effect of opiates, including morphine, on pathogenesis of HIV infections remains controversial. Opiates are well-known to modulate immune responses by preventing development of cell-mediated immune (CMI) responses. In this study using Simian Immunodeficiency Virus (SIV) macaque model of HIV pathogenesis, we undertook investigations aimed

exploring effects of morphine on neuropathogenesis and disease progression. Sixteen Rhesus macaques used in this study were divided into 2 groups; 4 administered saline, twelve others with morphine routinely 4 times a day. Both groups inoculated with SIVmacR71/17E and followed longitudinally for disease pathogenesis. The morphine group (M+V) exhibited higher mortality rates compared to virus alone (V) group. Interestingly, a subset of M+V animals succumbed to disease within weeks post-infection. These rapid progressors (RPs) exhibited higher incidence of end organ pathologies such as meningoencephalitis. Despite higher numbers of circulating CD4 and CD8T cells in M+V group the CD4:CD8 ratios remain unchanged in two groups. Viral loads in plasma and CSF of M+V group were at least a log higher than V alone group. Similarly there was trend of increased virus build-up in brains of M+V animals compared with V group. An intriguing finding of this study was increased numbers of monocyte/macrophages and virus in brains of M+V animals by immunohistochemistry, and in situ hybridization assays respectively. Supported by Grants DA020392 and DA024442 from the National Institutes of Health (SB).

Herpes Simplex Virus-1-Induced Reactive Oxygen Species Stimulate Cytokine Production in Murine Microglia. *S Hu*¹, WS Sheng¹, SJ Schachtele¹, JR Lokensgard¹; ¹Neuroimmunology Laboratory, Center for Infectious Disease, University of Minnesota Medical School, Minneapolis, MN 55455.

Production of reactive oxygen species (ROS) in the central nervous system contribute to neuronal damage during numerous pathological states. We have previously reported that non-productively infected microglial cells were the major source of inducible nitric oxide synthase during experimental murine herpes encephalitis. In the present study, oxidation of 2',7'-Dichlorofluorescein diacetate (DCFH-DA) was used to measure the production of intracellular ROS in cultures of primary murine microglia at 3, 8, 24, 48, and 72 h following infection with herpes simplex virus (HSV)-1. The levels of intracellular ROS were found to be highly elevated by 48 h post-infection (p.i.). Correspondingly, the majority of this virus-induced ROS production was blocked by diphenyleneiodonium (DPI), an inhibitor of NADPH oxidase. Interestingly, inhibition of NADPH oxidase also decreased virus-induced cytokine and chemokine production and phosphorylation of p38 MAP kinase. In addition, HSV was found to induce oxidative damage as assessed by 8-isoprostane levels. Finally, inhibitors of NADPH oxidase were found to block virus-induced apoptosis and oxidative damage. Taken together, these data demonstrate that HSV-induced cytokine and chemokine responses by microglia, as well as oxidative damage and apoptosis, are mediated through oxidative stress responses. Supported by NIMH/MH-066703.

Increased Ca²⁺ Influx and Pathophysiological Mechanisms Underlying the Excitotoxicity of HIV-1 Tat. *XT Hu*¹, L Al-Harhi¹, TC Napier¹; ¹Departments of Pharmacology, Immunology/Microbiology, Center for Compulsive Behavior and Addiction, Rush University Medical Center and Chicago D-CFAR, Chicago, IL 60612.

HIV expresses a number of verotoxins, including the HIV transactivator of transcription (Tat). Tat can cause dysfunction and degeneration of neurons and astrocytes in the brain, due in part to an excessive elevation of cytosolic Ca²⁺ to excitotoxic levels. Tat-induced excitotoxicity is associated with activation of NMDA-gated Ca²⁺ channels, but it is unknown if voltage-gated Ca²⁺ channels (VGCC) are also involved. Tat is damaging to the medial prefrontal cortex (mPFC), a critical brain region for cognition and addiction; thus, we hypothesized that VGCC are involved with Tat-induced excitotoxicity in mPFC. First, we employed electrophysiological approaches with rat mPFC slices to reveal that in pyramidal neurons, Tat facilitated firing and enhanced Ca²⁺ influx via L-type Ca²⁺ channels in a dose-dependent manner. The enhancements occurred even with blockade of NMDA receptors. Second, we used in vivo (intracerebroventricular) injections of Tat to show chronic astrocytosis (ie, increased GFAP) in rat mPFC ex vivo. Last, we used cultured astrocytes to reveal that Tat decreased active β -catenin, a central mediator of canonical Wnt signaling, which is a pro-survival pathway that is antagonized by excessive Ca²⁺ influx. The acute effects of Tat on neurons and astrocytes were abolished by selective blockade of L-channels. These findings revealed a novel pathophysiological mechanism underlying Tat excitotoxicity which could alter transmission from the mPFC to striatum/midbrain; and thus, may contribute to the neuropathogenesis of HIV-associated neurocognitive disorders. Supported by USPHSGs A-START DA-026746 to X-T.H. and D-CFAR P30AI082151.

Comparative Evaluation of Immune Cell Trafficking Across the Blood-Brain Barrier During Steady-State and Under the Neuroinflammation. *ZK Khan*¹, DS Sagar¹, EA Acheampong¹, SR Rahman¹, SM

Manuel¹, PJ Jain¹; ¹Drexel Institute For Biotechnology & Virology, Drexel University College of Medicine, Doylestown, PA 18902.

In the healthy individual infiltration of lymphocyte into the central nervous system (CNS) is very low and tightly controlled by the highly specialized blood-brain barrier (BBB). However, during neuroinflammation circulating lymphocytes readily cross the BBB and gain access to the CNS leading to edema, inflammation and demyelination. Interaction of circulating leukocytes with the endothelium of the BBB thus represents a critical step in the pathogenesis of neuroinflammatory diseases. To characterize the mechanisms of leukocyte trafficking, we have utilized a one-cell and a three-cell in vitro model of the BBB consisting of primary human brain-derived microvascular endothelial cells, neuronal cells and primary fetal astrocytes. Using these systems we investigated comparative trafficking of immune cells including dendritic cells (DCs), monocytes, CD4 and CD8 T cells under the steady state as well as during various inflammatory conditions. To this point, we have demonstrated that non-activated DCs (the most potent antigen presenting cells) exhibit a greater migratory potential compared to other immune cell tested during steady state. Activation of these cells enhances their trafficking across the BBB. Similarly, stimulation with the chemokine CCL2 or MCP-1 facilitate transmigration of both non-activated and activated leukocytes with DCs again being the top responding cell type. Our data contribute in the growing importance of DCs with respect to the neuroinflammation due to neurotropic infectious pathogens and/or autoimmune phenomenon such as multiple sclerosis. Supported by Drexel University College of Medicine.

Association of Dopamine Receptor D2 C957T Gene SNP in Learning and Memory Among HIV-Positive Alcohol Abusers. P Khatavkar¹, V Bryant¹, R Rosenberg¹, R Malow¹, J Devieux¹, Z Saiyed², S Thangavel², M Agudelo², M Nair²; ¹Stempel College of Public Health and Social Work, Florida International University, Miami, FL 33199, ²Institute of NeuroPharmacology, Herbert Wertheim College of Medicine, Florida International University, Miami, FL 33199.

Aim: People Living With HIV (PLWH) show neurocognitive deficits (e.g. memory, learning) which are secondary to cerebral dopaminergic degeneration and may promote unnatural reward seeking risky behaviors like hazardous drinking. Dopamine receptor D2 (DRD2) single nucleotide polymorphism (SNP) of C957T gene (T-minor allele) is found to have decreased up-regulation of DRD2 expression leading to decreased mRNA stability and translation to protein synthesis reflected in decreased dopaminergic neurocognitive functions. It is also associated with alcoholism and thus prompted to study DRD2-C957T SNP allelic discrimination and neurocognition among PLWH alcohol abusers. Methods: Seventy one participants (39 males, 32 females) of mean age 46 ±6.7 years were consented and classified as at Risk Drinkers (RD: AUDIT score ≤ 8) and Hazardous Drinkers (HD: AUDIT score > 17). Neurocognition was assessed using Auditory Verbal Learning Test (AVLT) and blood was drawn for allelic discrimination of the C957T gene SNP. Results: Genotype frequencies for C957T gene were TT (70%), CT (25%), CC (5%) with no significant age, gender, and racial differences. Significant differences were found among the C957T genotypes in relation to AVLT corrected total learning score (RD: $\chi^2=96.84$, $p=0.03$), proactive interference score (RD: $\chi^2=46.84$, $p=0.01$) and delayed recall score (RD: $\chi^2=104.90$, $p=0.04$), and learning rate (HD: $\chi^2=122.82$, $p=0.004$). Conclusions: Our results reveal that DRD2 C957T SNP which lowers dopaminergic neurocognitive capacities is associated with alcohol dependence in our study sample. Supported by RO1AA017405.

Ethanol-CYP3A4-Protease Inhibitors Three-Way Interactions: Implications for HAART Medications in Alcoholic HIV+ Individuals. S Kumar¹, M Jin¹, A Kumar¹; ¹Pharmacology and Toxicology, School of Pharmacy, University of Missouri-Kansas City, Kansas City, MO 64108.

CYP3A4 is the most abundant CYP in the liver and metabolizes ~50% of the drugs, including anti-retrovirals. Although CYP3A4 induction by ethanol and impact of CYP3A4 on drug metabolism and toxicity is known, CYP3A4-ethanol physical interaction and its impact on drug binding, inhibition, or metabolism is not known. Therefore, we studied the effect of ethanol on binding and inhibition of CYP3A4 with nine protease inhibitors (PIs). Our hypothesis is that ethanol shows differential PIs-CYP3A4 interactions. Nelfinavir, saquinavir, lopinavir, tipranavir, and atazanavir showed type I spectral binding, darunavir, amprenavir, and fosamprenavir showed low type I spectral binding, and indinavir and ritonavir showed type II spectral binding with CYP3A4. Subsequently, we measured binding affinity (KD and IC50), maximal spectral change (Amax), and maximal inhibition (Imax) for all the PIs with CYP3A4 in the presence of ethanol. Interestingly, ethanol decreased Amax, but did not alter binding affinity and Imax

with most of the type I PIs. However, it decreased binding affinity and I_{max} with low type I PIs. In contrast, ethanol increased the binding affinity without altering A_{max} and I_{max} with type II PIs. These results provide a model for differential ethanol-CYP3A4-PIs three-way interactions, which can be explained by physicochemical properties of the PIs and their binding characteristics with CYP3A4. This is an extremely important finding because alcoholism is prevalent in HIV-1-infected persons and alcohol is shown to differentially alter the response to HAART regimens. Supported by UMRB.

Modulation of Innate Immune-Related Pathways in Nicotine-Treated SH-SY5Y Cells. MD Li¹, WY Cui¹, JZ Ma¹, SL Chang²; ¹Department of Psychiatry and Neurobehavioral Sciences, University of Virginia, Charlottesville, VA 22911, ²Department of Biology, Seton Hall University, South Orange, NJ 07079.

Although nicotine has a broad impact on both the central and peripheral nervous systems, the molecular mechanisms remain largely unknown, especially at the signaling pathway level. To investigate that aspect, we employed both conventional molecular techniques, such as quantitative real-time PCR and Western blotting analysis, and a high-throughput microarray technique to identify the genes and signaling pathways modulated by nicotine. We found 14 pathways were significantly modulated by nicotine in SH-SY5Y neuroblastoma cells. Of these pathways, the Toll-like receptor (TLR; $p = 2.57 \times 10^{-4}$) pathway is one of the most important innate immune pathways. And death receptor (DR; $p = 8.71 \times 10^{-4}$) pathway, whose transducers coordinate TLR signals helping conduct host immune response to infection, was also significantly changed by nicotine. Furthermore, we found that many downstream pathways of TLR and DR signaling, such as PI3K/AKT ($p = 9.55 \times 10^{-6}$), p38 ($p = 2.40 \times 10^{-6}$), and ERK ($p = 1.70 \times 10^{-4}$), were also significantly modulated by nicotine. Interestingly, most of the differentially expressed genes in these pathways leading to nuclear factor kappa-B (NF- κ B) activation were up-regulated by nicotine, and the important inhibitors of pathways leading to apoptosis, including FLIP and Bcl-2, were also up-regulated. Taken together, our findings demonstrate that nicotine can regulate multiple innate-immune-related pathways. Thus, our data provide new clues to the molecular mechanisms underlying the contribution of nicotine to neuron survival. Supported by DA-013783; DA-016149; DA-026356.

HIV-1 gp120 Enhances Outward K⁺ Current and Resultant Neurotoxic Activity in Cultured Rat Microglia. J Liu¹, CH Xu¹, LN Chen¹, H Xiong¹; ¹Department of Pharmacology and Experimental Neuroscience, University of Nebraska Medical Center, Omaha, NE 68198-5880.

Microglia play an important role in the event of disease such as HIV-associated dementia (HAD). We hypothesize that an increase of outward K⁺ current (OKC) activates microglia, resulting in neuronal injury. To test this hypothesis, we studied the effects of HIV-1 gp120 on OKC in cultured rat microglia. Bath application of gp120 produced an enhancement of microglia OKC in a dose-dependent manner. The gp120-associated enhancement of OKC was blocked by T140, a CXCR4 receptor antagonist, suggesting the involvement of CXCR4 in gp120-induced enhancement of OKC. Further investigation showed that the gp120-induced enhancement of OKC was significantly blocked by H89, a specific protein kinase A (PKA) inhibitor, indicating that cAMP-dependent PKA signaling pathway is involved in gp120-associated increase of OKC. Studies on OKC biophysical properties revealed a slow inactivation mediated by gp120. Biological significance of gp120-induced enhancement of microglia OKC was demonstrated by experimental results that the cytotoxic activity of gp120-stimulated microglia, evaluated by TUNEL staining and MTT assay, was blocked by K⁺ channel blockers. Taken together, these results suggest that brain infection of HIV-1 may induce microglia cytotoxic activity by enhancing OKC and that microglia K⁺ channels could be potential targets for the development of therapeutic strategies. Supported by NINDS 2R56NS041862-10.

HIV-1 Tat Protein Expression in Mouse Brain Potentiates the Psychostimulant Effects of Cocaine and Reinstates an Extinguished Cocaine Craving. JP McLaughlin¹, CF Shay², SM Gomes², AM Carey²; ¹Department of Biology, Torrey Pines Institute of Molecular Studies, Port St. Lucie, FL 34987, ²Department of Psychology, Northeastern University, Boston, MA 02115.

While the HIV-1-accessory protein Tat is known to synergize with psychostimulant drugs to exacerbate neurotoxicity, the functional consequences of Tat protein on the behavioral response to abused drugs are little known. Accordingly, we hypothesized that HIV-1 Tat expression in brain would modulate the psychostimulant effects of cocaine. Using the GT-tg bigenic mouse model, where brain-selective Tat expression is induced by activation of a doxycycline (Dox) promoter, we tested the effects of Tat protein on cocaine-induced locomotor activity and conditioned place preference (CPP). Tat-induced GT-tg

bigenic mice demonstrated a significant increase in the locomotor effects of cocaine (10 mg/kg, s.c.), but not saline, over the responses of uninduced GT-tg littermates and Dox-treated C57Bl/6J mice. Moreover, although GT-tg bigenic mice expressing Tat demonstrated saline-conditioned place preferences similar to uninduced littermates and Dox-treated C57Bl/6J mice, Tat expression significantly increased cocaine-CPP 3-fold. Consistent with this observation, cocaine place-conditioned GT-tg bigenic mice subsequently made to express Tat protein demonstrated a significant increase in cocaine-CPP after an additional cycle of cocaine place conditioning as compared to the response of uninduced littermates. Of interest, subsequent exposure to Tat protein resulted in the reinstatement of an extinguished cocaine-CPP in previously uninduced mice. Overall, these data suggest that expression of HIV-1 Tat protein in mouse brain potentiated the psychostimulant effects of and craving for cocaine. Supported by the State of Florida.

HIV-1 Tat Protein Decreases Dopamine Transporter Cell Surface Expression and Vesicular Monoamine Transporter-2 Function. *NM Midde*¹, *AM Gomez*¹, *J Zhu*¹; ¹Department of Pharmaceutical and Biomedical Sciences, University of South Carolina, Columbia, SC 29208.

The dopamine (DA) transporter (DAT) and vesicular monoamine transporter (VMAT2) proteins interact as a biochemical complex to regulate dopaminergic neurotransmission. We have reported that HIV-1 Tat1-86 decreases the specific [³H]DA uptake and [³H]WIN 35,428 binding sites without a change in total DAT immunoreactivity in rat striatum (Zhu et al., 2009). In the present study, the effects of Tat on DAT trafficking and vesicular [³H]DA uptake in rat striatum were investigated. Rat striatal synaptosomes were incubated with or without Tat protein (1 μM). Subsequently, the subcellular fractions, total synaptosomal fractions (P2), plasma membrane enriched fractions (P3) and vesicle-enriched fractions (S3), were prepared for Western blotting and [³H]DA uptake assays. The DAT immunoreactivity was greater in P3 than in S3, suggesting that cell surface DAT expression is modulated by endocytotic trafficking. Tat decreased DAT expression in P3 fractions, accompanied by an increase of DAT in S3 fractions. In addition, Tat inhibited the specific [³H]DA uptake into vesicles (S3) and synaptosomes (P2) by 35% and 26%, respectively. These results suggest that the Tat-induced decrease in cell surface DAT expression is responsible for the decrease in DAT function. Although both DAT and VMAT2 proteins are essential for the regulation of DA disposition in synapse and cytosol, the inhibitory effect of Tat was more profound in VMAT2 protein than in DAT protein, suggesting that VMAT2 protein plays a more critical role in Tat-induced alterations of extracellular DA concentrations. Supported by DA026721.

Systems Biology Analysis of Gut-Specific Mechanisms Underlying Chronic Δ-9-THC Modulation of Simian Immunodeficiency Virus Infection. *PE Molina*¹, *NJ LeCapitaine*¹, *J Zabaleta*¹, *A Amedee*¹, *P Zhang*¹, *P Winsauer*¹; ¹Physiology/School of Medicine, Louisiana State University Health Sciences Center, New Orleans, LA 70112.

We demonstrated that chronic Δ-9-tetrahydrocannabinol (THC) administration attenuates viral load and disease progression in simian immunodeficiency virus (SIV)-infected rhesus macaques. Gut-associated lymphoid tissue is an important site for HIV replication and inflammation and is a possible site for THC immunomodulation. A systems approach was used to examine the impact of chronic THC administration (0.18-0.32 mg/kg i.m., 2 X daily), starting 12 months prior to inoculation with SIVmac251 (100 TCID₅₀/ml, i.v.), on gastrointestinal mucosa (GI) viral load, lymphocyte phenotype, and transcriptome at ~5 mo post-inoculation. GI viral load was lower in THC/SIV than in VEH/SIV (2.04 ± 0.41 vs 3.05 ± 0.68 RNA Log copies/ng tissue mRNA) macaques. 115 genes were differentially expressed (53 up- & 62 down-regulated) in GI mucosa samples of THC/SIV when compared to those of VEH/SIV macaques. A total of 12 pathway maps were identified to include 3 or more differentially expressed genes (p<0.05). Of these, the predominant cell processes were involved in cytoskeleton remodeling (45%), immunomodulation (27%), cell adhesion/integrin (18%), and cell signaling (9%). GI mucosa of THC/SIV macaques had significantly (p<0.05) more CD8 (28%) and CD4 (51%) integrin β7 positive central memory lymphocytes than VEH/SIV animals (19% and 5%, respectively). These findings suggest that the mechanisms involved in chronic THC modulation of SIV/HIV's disease progression result from structural and functional modification of GI barrier function and lymphocyte phenotype, as well as suppressed GI viral replication. Supported by NIDA/1R01DA020419-05, NIDA/ 3R01DA020419-04S1, NIDA/1R01DA030053-01.

Attenuation of High-Fat-Diet-Induced Neuroinflammation by Momordica Charantia. PV Nerurkar¹, LM Johns¹, LM Buesa¹, G Kipyakwai¹, E Volper², R Sato¹, P Shah¹, VR Nerurkar²; ¹College of Tropical Agriculture & Human Resources, University of Hawaii, Honolulu, HI 96822, ²John A. Burns School of Medicine, University of Hawaii, Honolulu, HI 96813.

Rising epidemic of obesity is considered a major risk factor for neurodegenerative diseases. Increased metabolic flux during overnutrition and obesity can orchestrate stress response, blood-brain barrier (BBB) disruption, recruitment of inflammatory immune cells from peripheral blood and microglial cells activation leading to neuroinflammation. The lack of an effective treatment for obesity-associated brain dysfunction may have far-reaching public health ramifications urgently necessitating the identification of appropriate preventive and therapeutic strategies. The objective of our study was to investigate the neuroprotective effects of Momordica charantia (bitter melon, BM) on high-fat diet (HFD)-associated BBB permeability, stress and neuroinflammatory cytokines. C57BL/6 female mice were fed HFD with and without BM for 16 weeks. BBB permeability was analyzed using Evans blue dye. Phosphate buffered saline (PBS) perfused brains were analyzed for neuroinflammatory markers such as interleukin-22 (IL-22), IL-17R, IL-16, NF- κ B, as well as antioxidant enzymes using microarray, quantitative real-time RT-PCR and enzymatic assays. Our data indicates that BM ameliorated HFD-associated changes in BBB permeability as evident by reduced leakage of Evans blue dye. HFD-induced neuroinflammatory markers were normalized in the brains of mice supplemented with BM. Similarly, HFD-induced brain oxidative stress was significantly reduced by BM supplementation. Functional foods such as BM may offer a unique therapeutic strategy to improve obesity-associated neuroinflammation. Supported by NCCAM (R21AT003719), RCMI, NCRR (G12RR003061), NIH.

Possible Role of GPR55 in the Cannabinoid-Induced Increase in Serum IgE in Mice. C Newton¹, C Patterson¹, T Klein¹; ¹Molecular Medicine, University of South Florida College of Medicine, Tampa, FL 33612.

During the development of adaptive immunity to Legionella pneumophila infection, Δ -9-tetrahydrocannabinoid (THC) induces a shift from cell-mediated Th1 responses to humoral Th2 responses and this involves CB1 and CB2 cannabinoid receptors. In in vivo IgE induction models using OVA/ALUM or KLH/RIBI, we studied the role of another cannabinoid receptor, GPR55A, in cannabinoid effects on antibody production. BALB/c (B/c), C57BL/6 (B6), or B6 CB2 deficient (CB2^{-/-}) mice were pretreated with THC or L- α -lysophosphatidylinositol (LPI) prior to injections with OVA/ALUM or KLH/RIBI followed by boosting with same antigens. The mice were then bled on various days after boosting and total serum IgE was determined. The IgE levels were elevated in THC treated B/c mice in response to either OVA/ALUM or KLH/RIBI. Interestingly with OVA/ALUM, CB2^{-/-} mice had increased levels of IgE over wildtype (B6) mice and pretreatment with THC augmented this effect. CB1 antagonist treatment of B6 and CB2^{-/-} mice prior to THC dosing slightly elevated the IgE response suggesting neither CB1 nor CB2 were involved in the IgE response. With KLH/RIBI, THC treatment increased IgE levels greater than OVA/ALUM; however, there were no differences between B6 and CB2^{-/-} or THC/B6 and THC/CB2^{-/-} mice. Pretreatment with the GPR55-selective agonist LPI, enhanced IgE levels in B/c mice given OVA/ALUM. These studies suggest that GPR55 is involved in enhancing IgE production following treatment with THC and LPI. Supported by NIH grant DA019824.

HIV-1 LTR Single Nucleotide Polymorphisms Correlate with Use of Drugs of Abuse in the DrexelMed HIV/AIDS Genetic Analysis Cohort. MR Nonnemacher¹, B Aiamkitsumrit¹, V Pirrone¹, A Wojno¹, S Passic¹, B Blakey¹, J Ku¹, N Parikh¹, B Moldover³, R Feng⁴, L Servance², D Downie², S Lewis², J Jacobson², B Wigdahl¹; ¹Department of Microbiology and Immunology, Drexel University College of Medicine, Philadelphia, PA 19102, ²Division of Infectious Disease and HIV Medicine, Drexel University College of Medicine, Philadelphia, PA 19102, ³B-Tech Consulting, Ltd, Philadelphia, PA 19104, ⁴Department of Biostatistics and Epidemiology, University of Pennsylvania, Philadelphia, PA 19104.

HIV infection is prevalent among substance abusers. The effects of specific illicit drugs on HIV-1 disease progression have not been well established. We evaluated the relationship between illicit drug use and HIV-1 disease progression in 418 HIV-1-infected patients enrolled in the DREXELMED HIV/AIDS Genetic Analysis Cohort in Philadelphia, PA. History of illicit drug, alcohol, and medication use, CD4+ and CD8+ T cell count, and viral load were performed approximately every 6 months. Drug abuse was common in the cohort with 55% of patients admitting past use, 31% current drug abuse, and 27% testing positive for drug use at the time of visit. Cocaine and marijuana were heavily favored, with 82% of

patients admitting to past or current cocaine use and 72% admitting to marijuana use. The cohort currently contains a total of 30 non-users, 70 cocaine only (preferential) users, and 36 cannabinoid only (preferential) users. Non-users were more likely to remain on ART (90%), while the cocaine- and cannabinoid-only are less likely to remain on ART (67% and 78%, respectively). Drug users had both a lower current CD4+ T cell count as well as lower nadir CD4+ T cell and higher current viral loads and higher peak viral loads as compared to non-users. In addition, single nucleotide polymorphisms (SNPs) were identified that are unique to cocaine, marijuana, or non-users. In conclusion, illicit drug use appears to facilitate HIV-1 disease progression. In addition, use of drugs of abuse selects for genetic variations unique to mono- and multi-using HIV/AIDS patient cohorts. Supported by R01 NS032092, R01 DA019807, T32 MH079785.

Inhibition of Antibody Class Switching to IgE By siRNA Targeting Cannabinoid Receptors. *CE Patterson*¹, C Newton¹, TW Klein¹; ¹Department of Molecular Medicine, University of South Florida, Tampa, FL 33612.

Work in our laboratory has shown that cannabinoid treatment of primary mouse B cells results in antibody class switching favoring increased IgE production. IgE expression is one hallmark of a Th2 immune response that can be directly linked to many allergic conditions. Use of CB2-KO mice, a non-selective cannabinoid receptor agonist, CP55940, and selective agonist for CB1 showed the dependence on CB2 for this Th2-biased response both in vivo and in vitro. Immune cells express CB2, with B cells expressing it the most. We discovered that CB2 expression in mouse cells occurs through three different transcriptional start sites. We show here that siRNA to CB2 receptor message indeed knocks down RNA levels (qPCR) as well as total protein levels (ICC flow cytometry). Targeting the coding region reduces these levels more than specifically targeting one of the three unique 5'-UTR regions, as expected. Currently we are evaluating the inhibitory potential of siRNA in the suppression of IgE production from B cells following stimulation with the CB2 receptor agonists. Lowering IgE levels by suppressing CB2 receptor expression could be a valuable tool to manage allergic disease. Supported by NIDA grant DA19824.

Deciphering Synaptic Perturbations During HIV/METH CNS Dysfunction. *G Pendyala*¹, HS Fox¹; ¹Department of Pharmacology & Experimental Neuroscience, University of Nebraska Medical Center, Omaha, NE 68198.

HIV and drugs of abuse have a substantial effect on the CNS and in synergy have been implicated to increase cognitive impairments. In order to investigate mechanistic clues to the effects of substance abuse at the synapse, we performed proteomics profiling on synaptosomes isolated from the caudate nucleus of SIV infected and methamphetamine (METH) treated nonhuman primate model for AIDS. Among the differential synaptic fingerprints identified, we focused on one protein Na⁺/K⁺ ATPase alpha 3 subunit (ATP1A3) was upregulated in the SIV and METH treated group compared to the SIV only. ATP1A3 aids in active transport of the Na⁺ and K⁺ ions across the cell plasma membrane and maintains the chemical gradients of these ions via hydrolysis of adenosine triphosphate (ATP). Emerging studies have also demonstrated signal transducing ability for ATP1A3 specifically acting via mitogen activated protein kinases (MAPK). Treatment of primary rat neurons with METH revealed an up regulation showing that independent of SIV, there is a direct effect of METH to increased neuronal ATP1A3 expression. An interesting observation was a sustained increase in extracellularly regulated kinases 1/2 (ERK1/2) phosphorylation until 30 min after METH treatment. These parallels, while indirect, point to one novel mechanism of action of METH in the CNS is to interact with ATP1A3 and elicit signaling responses. Current studies are focused on deciphering a possible ligand-receptor relationship and associated results will be presented at the meeting. Supported by NIH grants P30 MH062261, R01 MH073490 and P01DA026146 (HSF).

Activation of the Antioxidant Defensive Enzymes Against HIV-1 gp120 are Mediated by Oxidative Stress and Elevated Intracellular Calcium Levels. *VB Pichili*¹, Z Saiyed¹, T Samikkannu¹, M Agudelo¹, N Gandhi¹, A Yndart¹, MP Nair¹; ¹Department of Immunology, Institute of NeuroImmune Pharmacology, Florida International University, Miami, FL 33136.

HIV infection has been shown to significantly affect the functioning of the central nervous system leading to HIV associated neurocognitive disorder (HAND) which is characterized by depression, behavioral and motor dysfunctions. The viral envelope protein, gp120 is known to stimulate the release of

neurotoxic factors thereby causing the apoptotic cell death. HIV virus, and its viral components TAT, gp120 have been demonstrated to elevate the generation of reactive oxygen species and induce oxidative stress. We hypothesize that the oxidative stress caused by the HIV-1 gp120 protein induces the expression of antioxidant defensive enzymes Hemoxygenase-1 (HO-1) and NADPH quinone oxidoreductase (Nqo1) in cultured astrocytes. Cultured astrocytes were treated with gp120 protein for different time periods, RNA was isolated, reverse transcribed and analyzed by qRT-PCR. Total cell lysates were analyzed using western blots to assess the protein expression. gp120 upregulates the expression of gene and protein levels of Hemoxygenase-1, and NAD(P)H dehydrogenase quinone1 in human astrocytes. Suppression of the reactive oxygen species by pretreating with antioxidants attenuates the increased expression of HO-1 and Nqo1. Further, inhibitor of calcium also prevented the increased expression of HO-1 and Nqo1 implicating the role of elevated calcium in HAND. Our results suggest that the astrocytes upregulate their antioxidant defense mechanisms in response to the gp120 induced oxidative stress and the consequent elevation of intracellular calcium levels. Supported by RO1DA012366, RO1DA021537 and R37DA025576.

Methamphetamine Alters T Cell Cycle Progression: Role in Immune Dysfunction. *R Potula*¹, J Cenna¹, S Fan¹; ¹Pathology and Laboratory Medicine, Temple University School of Medicine, Philadelphia, PA 19140.

The underlying mechanisms secondary to methamphetamine (METH) abuse, in particular T cell responses, remains largely unknown and warrants further consideration. Our earlier studies have demonstrated that METH exposure results in the loss of T cell functions (IL-2 production and T cell proliferation). The current study was carried out to gain new insights into the effects of METH on T cell cycle entry and progression. Results obtained from cell cycle analysis indicated that compared to the controls, significant number of T cells exposed to METH remain arrested in G₀/G₁ phase suggesting that METH modulate the normal cell cycle. Genomic analysis of cell cycle performed to determine the effects of METH on cell cycle exit of T lymphocytes showed differentially regulated genes: that both positively and negatively regulate the cell cycle, associated with transitions between the each of the phases, DNA replication, checkpoints and arrest. Taken together, our results provide reasonable evidence for our hypothesis that METH exposure results in altered cell cycle entry and progression. Studies are underway to examine if tainted expression of cell cycle progression markers (cyclins and the cyclin-dependent kinases) are contributory to the impaired cell cycle progression of METH exposed T cells. Supported by NIDA / R01 DA031064.

Cannabinoid Receptor Expression in Primary Human Microglia: The Role of CB2. *RB Rock*¹, S Hu¹, WS Sheng¹, PK Peterson¹; ¹Center for Infectious Diseases and Microbiology Translational Research, University of Minnesota, Minneapolis, MN 55455.

Cannabinoid receptors (CBs) in the CNS play an important role in modulating brain development and inflammatory responses. We and others have found that CB2 is expressed on microglial cells. Activation of CB2 receptors have been reported to regulate many physiological functions, therefore CB2 is considered a therapeutic target. In this study we demonstrated CB expression in human primary microglia obtained from different brain specimens. We found that often the CB1/CB2 ratios were variable in resting microglial cells. The specific binding of 3H-CP55,940 was around 55-70%. Using selective CB antagonists, SR141716A and SR144528, displacement of 3H-CP55,940 binding was observed demonstrating both CB1/CB2 receptor expression. Human primary microglia express CCR2 receptors, enabling the cells to migrate toward CCL2 in a concentration-dependent manner. Pretreatment with CB ligands (WIN55,212-2, CP55,940 and JWH015) downregulated microglial migration toward CCL2 and CB2 selective antagonist SR144528 significantly reversed the inhibitory effect of WIN55,212-2, suggesting a CB2-mediated mechanism. No effect of CB ligand treatment on CCR2 expression was found indicating that migratory inhibition was not due to CCR2 expression change. Using real-time PCR, we found that CB1 mRNA expression was relatively unaffected by several cytokine, bacterial and viral products. However, CB2 expression was markedly down-regulated by products such as LPS and Tat. These findings suggest that CB2 expression can be regulated by external stimuli, which has important therapeutic implications. Supported by NIDA/DA025525.

Tobacco Cembranoid 4R Attenuates HIV Neurotoxicity by Glutamate Release Reduction Independent of Viral Replication and Inflammation. *JW Rodriguez*¹, M Rodriguez-Martinez¹, PA

Ferchmin¹, E Rios-Olivares¹, D Wang², A Nath², VA Eterovic¹; ¹Department of Microbiology and Immunology, Universidad Central del Caribe School of Medicine, Bayamon, PR 00960, ²Department of Neurology, Johns Hopkins University School of Medicine, Baltimore, MD 21287.

HIV-associated neurocognitive disorders (HAND) has become the most common neurologic complication of AIDS, affecting approximately 50% of HIV-infected patients. There is a major need to identify a compound that can prevent or alleviate the damaging effects following HIV-1 infection in the brain. Our laboratory has identified a non-toxic compound from the tobacco leaf called cembranoid 4R that readily penetrates into the brain. Using PBMC and glial cell lines as a test model, RT-PCR for HIV-1 replication, cytometric bead array (CBA) for inflammatory cytokine production, glutamate assay, and MTT were performed. We show that 4R suppressed HIV-1 replication in PBMC by 4-fold, but that was not the case in human glial cells which 4R enhanced it by 2-fold. However, PBMC secretions attenuated HIV-1 infection in glial cells in the presence of 4R using a transwell system. We also demonstrate that 4R downregulated inflammatory cytokines such as IL-1 β and TNF- α in PBMC whereas it upregulated inflammatory cytokines such as IL-6, IL-8 and MCP-1 in human glial cells. In addition, 4R attenuates glutamate release and reverse significantly the neurotoxicity induced by HIV-infected supernatants. Neuroprotection by 4R occurs independently of viral load levels and inflammatory cytokines release. This study reveals new properties of the novel compound 4R and provides new understanding of the mechanisms by which this compound may elicits its properties. Further knowledge of the mechanism by which 4R exerts neuroprotection could lead to the development of therapy for treating HAND. Supported by NIH/NIMH grant 5R25MH0806603 and RCMI grant G12RR03035.

MicroRNA Profiling in Human Brain Microvascular Endothelial Cells after Inflammatory Insult and Inhibition of Glycogen Synthase Kinase 3 β (GSK3 β). S Rom¹, S Fan¹, S Dykstra¹, N Reichenbach¹, S Ramirez¹, Y Persidsky¹; ¹Department of Pathology and Laboratory Medicine, Temple University School of Medicine, Philadelphia, PA 19140.

Immune mediators and BMVEC-leukocyte engagement contribute to blood brain barrier (BBB) impairment during neuroinflammation. We identified GSK3 β as a potent regulator of immune responses in vitro BBB system and animal model. Inflammatory response induces several hundred genes, a process that is tightly regulated to prevent the consequences of unregulated expression. miRNAs have emerged as gene expression regulators. The relationship between inflammation and miRNA expression remains largely is unexplored. We used microarray technology (miR Base version 16 screening of 1212 miRNAs) to identify miRNAs induced in human primary BMVEC after exposure to pro-inflammatory cytokine, TNF- α with or without GSK3 β inhibitor, LiCl miRNA array showed that 123 microRNAs were downregulated after TNF-alpha exposure (< -1-fold), and 193 microRNAs to be upregulated (>1-fold) in cells treated by TNF- α /LiCl. 14 miRNAs were common for two groups. qPCR confirmed microarray results for 3 miRNAs with largest fold change (mir-98, mir-629* and let-7g*). Treated with TNF- α of the immortalized human brain endothelial cell line D3 also resulted in down regulation of the same miRNAs, and GSK3 β inhibition partially reversed TNF- α effects. Among the top ranking predicted targets of 3 miRNAs are several inflammatory molecules such as VCAM-1, CD99, L1CAM, IL8, IL6, CXCL1 and IP-10. Our previous studies indicated VCAM-1, IL8, IP-10 and CXCL1 were upregulated by TNF- α in BMVEC and suppressed by GSK3 β inhibitors. We are in process of further validation of these results by overexpression of miRNAs in BMVEC or D3 cells. Supported by AA015913, MH65151.

Differential Regulation of Neuroplasticity Associated Genes by HIV-1B and C Clades. ZM Saiyed¹, NH Gandhi¹, J Napuri¹, A Yndart¹, M Agudelo¹, VB Pichili¹, T Samikkannu¹, MN Nair¹; ¹Immunology, Institute of NeuroImmune Pharmacology, College of Medicine, Florida International University, Miami, FL 33199.

Experimental and clinical evidence suggests difference in manifestation of HAND between clade B and C-infected subjects with clade B being more neuropathogenic than clade C. However, the exact mechanism underlying the differences in the neuropathogenesis by both the subtypes remains elusive. Previous studies with HIV-1B have implicated that HIV-induced neurocognitive deficits could be due to dysregulation of genes such as CREB, CaMKIIa that are known to regulate neuronal plasticity. Recently, it was also shown that MEF2C, a neurogenic transcription factor that promotes expression of ARC and SynGap was downregulated in the brain of SIV infected macaques. We therefore hypothesize that clade B and C exert differential effects on CNS cells leading to differential expression of genes that regulate synaptic plasticity and neuronal activity. Primary human astrocytes were separately infected with HIV-1

clade B and C virus and the RNA was extracted, followed by quantitative real time PCR for CREB, MEF2C and ARC genes and the cell lysate was tested for protein levels by western blot. Results indicate that HIV-1B significantly downregulated expression of synaptic plasticity genes, CREB, MEF2C and ARC by primary astrocytes compared to HIV-1C infected cultures. This suggests that infection with HIV-1B and C virus differentially modulates the expression of genes involved in synaptic plasticity and neuronal function. The results emanating from these studies may elucidate the differential mechanisms of clade specific infection in development of HAND and may be of therapeutic significance. Supported by NIMH.

Differential Regulation of Thiol Modification by HIV-1 Clade B and C Protein. *T Samikkannu*¹, *Z Saiyed*¹, *M Agudelo*¹, *PV Reddy*¹, *D Nwankwo*¹, *N Gandhi*¹, *P Khatavkar*¹, *Y Yndart*¹, *MP Nair*¹;
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Aim: Previous studies have demonstrated that infections with HIV-1 clades differentially contribute to the neuropathogenesis of HIV-associated neurocognitive disorder (HAND). The thiol modification leads to down regulation of rate limiting enzyme glutathione (GSH) which is known to play a significant role in neuropathogenesis of HAND. We hypothesize that clade B and C Tat proteins exert differential effects on human primary neurons by down regulation of glutathione synthetase (GSS) and altered GSH/GSSG. **Methods:** RNA extracted from human primary neurons treated with HIV-1 clade B and C Tat proteins was reverse transcribed and analyzed by quantitative real-time PCR to determine GSS gene expression and the cell lysates were analyzed for GSH/GSSG levels. **Results:** Our results indicate that HIV-1 clade B gp120 protein significantly down regulated GSS gene expression, and altered GSH/ GSSG compared with clade C Tat protein. **Conclusions:** Thus, our studies for the first time demonstrate that HIV-1 clade B Tat protein appears to significantly down regulate GSS gene expression and altered GSH/GSSG as compared to HIV-1 clade C Tat protein. This suggests a differential effect of HIV-1 clade B leading to increased neuropathogenesis and associated HAND in HIV-1B infected subjects compared to HIV-1C infected subjects.

Inhibition of Reactive Oxygen Species Production from Activated Human Astrocytes by Synthetic Cannabinoids. *W Sheng*¹, *S Hu*¹, *A Feng*¹, *PK Peterson*¹, *RB Rock*¹;
¹Center for Infectious Diseases and Microbiology Translational Research, University of Minnesota Medical School, Minneapolis, MN 55455.

Reactive oxygen species (ROS) have been shown to be a contributor to aging and disease. ROS also serves as trigger switch for signaling cascades leading to corresponding cellular and molecular events. In the central nervous system microglial cells are likely the main source of ROS production. However, activated astrocytes have also been reported to produce ROS. The anti-inflammatory and neuroprotective properties of cannabinoids have been reported to involve cannabinoid receptor- or non-receptor-mediated mechanisms, and the expression of CB1/CB2 receptors has been demonstrated in astrocytes. In this study we investigated the effect of cannabinoid ligands on ROS production by human brain astrocytes stimulated with IL-1 β and interferon (IFN)- γ using H2DCFDA to assess ROS generation. Although IFN- γ alone had minimal effect, it potentiated IL-1 β -induced ROS in a time-dependent manner. Pretreatment with DPI (diphenylene iodonium), an inhibitor of NADPH oxidase, blocked the IL-1 β /IFN- γ -induced ROS production effectively. Meanwhile, the antioxidant enzyme mRNA expression such as glutathione reductase and catalase were inhibited and superoxide dismutase-2 enhanced by IL-1 β but not IFN- γ . When pretreated with cannabinoid ligands downregulation of IL-1 β /IFN- γ -induced ROS production was observed in astrocytes. Our results support the notion that synthetic cannabinoids have anti-oxidant property and that they may have therapeutic potential for ROS-induced damages.

Lipopolysaccharide Differentially Regulates MDR1 and MRP1 in Human Macrophages. *PS Silverstein*¹, *A Kumar*¹;
¹Division of Pharmacology and Toxicology, School of Pharmacy, University of Missouri-Kansas City, Kansas City, MI 64108.

Multidrug resistance-associated protein 1 (MRP-1, ABCC1) and P-glycoprotein (MDR1, ABCB1) are both members of the ATP-binding cassette transporter family. Both of these proteins transport antiretroviral therapeutics, such as HIV-1 protease inhibitors (PI). Previous reports have demonstrated that the intracellular concentrations of HIV-1 protease inhibitors may be below their EC50 or EC90 in chronically infected macrophages. Our hypothesis is that inflammation will alter the expression and function of these transporters in macrophages. We treated THP-1 macrophages to LPS and determined the expression levels of both MRP1 and MDR1 in these cells. Our results demonstrate that at the levels

of mRNA, protein, and functional activity, LPS increased MRP1 expression and decreased the expression of MDR1. These results indicate that the effectiveness of antiretroviral therapy may be affected by inflammation and opportunistic infections. Furthermore, the differential regulation of MDR1 and MRP1 that was observed suggests that the effects of LPS on the intracellular concentration of protease inhibitors in macrophages may be specific for each PI. Supported by NIH.

HIV Induce Differential Interclade Human Neuronal Responses by Proteomic Fingerprinting. *K Trujillo-Nevarez*¹, G Gonzalez¹, L Cubano¹, E Rios-Olivares¹, JW Rodriguez¹, NM Boukli¹; ¹Department of Microbiology and Immunology, Universidad Central del Caribe, Bayamon, PR 00960.

It is suggested that the degree of neuroAIDS vary according to the HIV-1 clade, but the molecular mechanisms behind the differences still remain unclear. Exploiting proteomics, we hypothesize that HIV-1 clade B and C induce differential protein profiles on human neurons. Proteins from neurons treated with HIV-1 clade A and B were separated with 2 dimensional gel electrophoresis, followed by mass spectrometry to establish homologies and dissimilarities in protein expression. 2D maps of the clade B and clade C 2D maps indicated that a total of 164 and 214 proteins were modulated with HIV-1 clade B and clade C treatments respectively compared to 325 proteins control. Our results suggest that among differentially expressed proteins, HIV-1 clade B upregulates significantly (1) a BRCA1-associated RING domain protein involved in neuronal injury and apoptosis, (2) a serotransferrin involved in oxidative stress defense and, (3) a ketohexokinase (fructokinase) that play an important role in the epidemic of metabolic syndrome as compared to HIV clade C. In addition, HIV-1 clade C significantly upregulates the expression of (1) ankyrin which is associated with the capacity to block HIV entry and, (2) cysteine sulfenic acid (the initial product of oxidation of cysteine) by cellular ROS as compared to HIV clade B. Taken together, the data suggest that HIV-1 clade B appears to specifically induce key markers of HIV-1 pathogenesis as compared to HIV-1 clade C which upregulated molecules mainly involved in protein oxidation and in the suppression of HIV-1 entry. Supported by NIH-RCMI Biomedical Proteomics Facility 2G12RR03035.

Modulation of Intracellular Restriction Factors Contributes to Methamphetamine-Mediated Enhancement of AIDS Virus Infection of Macrophages. *X Wang*¹, YZ Wang¹, L Ye¹, JL Li¹, L Song¹, N Fulambarkar¹, WZ Ho¹; ¹Department of Pathology and Laboratory Medicine, Temple University School of Medicine, Philadelphia, PA 19140.

Epidemiological studies have demonstrated that the use of methamphetamine (METH), a sympathomimetic stimulant, is particularly common among patients infected with HIV. In vitro studies also have determined that METH enhances HIV infection of CD4+ T cells, monocyte derived dendritic cells, and macrophages. In addition, animal studies have showed that METH treatment increases brain viral load of SIV infected monkeys and promotes HIV production and viremia in HIV/hu-CycT1 transgenic mice. However, the mechanism(s) of these actions have not been well determined. In this study, we investigated the impact of METH on intracellular restriction factors against HIV and SIV. We demonstrated that METH treatment of human blood mononuclear phagocytes significantly inhibited the expression of anti-HIV microRNAs and several key elements (RIG-I, IRF-3, SOCS-2, 3 and PIAS-1, 3, X) in type I IFN pathway. The suppression of these innate restriction factors was associated with reduced production of type I IFNs and the enhancement of HIV or SIV infection of macrophages. These findings indicate METH use impairs intracellular innate antiviral mechanism(s) in macrophages, contributing to cell susceptibility to AIDS virus infection. Supported by NIH DA12815, DA22177 and DA27550.

TRPC Channel-Mediated Neuroprotection by PDGF Involves Pyk2/ERK/CREB Pathway. *HH Yao*¹; ¹Pharmacology, University of Nebraska Medical Center, Omaha, NE 68198.

Platelet-derived growth factor-BB (PDGF) has been reported to provide tropic support for neurons in the central nervous system. The protective role of PDGF on dopaminergic neurons, especially in the context of HIV-associated dementia (HAD), however, remains largely unknown. Herein we demonstrate that exogenous PDGF was neuroprotective against toxicity induced by HIV-1 Tat in primary midbrain neurons. Furthermore, we report the involvement of transient receptor potential canonical (TRPC) channels in PDGF-mediated neuroprotection. TRPC channels are Ca²⁺-permeable, nonselective cation channels with a variety of physiological functions. Blocking TRPC channels with either a blocker or short interfering RNAs (specific for TRPC 5 and 6) in primary neurons resulted in suppression of both PDGF-mediated neuroprotection as well as elevations in intracellular Ca²⁺. PDGF-mediated neuroprotection

involved parallel but distinct ERK/CREB and PI3K/Akt pathways. TRPC channel blocking also resulted in suppression of PDGF-induced Pyk2/ERK/CREB activation, but not Akt activation. Relevance of these findings in vivo was further corroborated by intrastriatal injections of PDGF and HIV-1 Tat in mice. Administration of PDGF was able to rescue the dopaminergic neurons in the substantia nigra from Tat-induced neurotoxicity. This effect was attenuated by pre-treatment of mice with the TRP blocker, thus underscoring the novel role of TRPC channels in the neuroprotection mediated by PDGF. Supported by NIH.