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SYMPOSIA

The 25th Scientific Conference of the Society on Neuroimmune Pharmacology: Program and Abstracts

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Guarantor: Marcus Kaul

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Abstract
The 25th Scientific Conference of the Society on Neuroimmune Pharmacology (SNIP) will take place in Portland, OR, USA, from April 10 - 13, 2019. Over four days, the conference will provide insights into the latest and most advanced science in the intersecting areas of neuroscience, immunology, pharmacology and its translational aspects, in particular drug abuse. Abstracts are ordered in three major groups: 1) speakers, 2) poster presenters Wednesday (W01-52) and 3) poster presenters Thursday (T01-52).

Keywords: Brain; Viral Infection; HIV; Drug abuse; Alcohol; Behavior; Pharmacology; Immunology; Neuroscience

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Program and Abstracts of the 25th SNIP Scientific Conference

Program

Wednesday April 10, 2019

SNIP Council Meeting	10:00 AM – 12:00 PM
Registration for SNIP Meeting Open	2:00 – 8:00 PM
Satellite Symposium	12:00 – 6:00 PM
Unraveling NeuroAIDS in the Presence of Substance Use Disorder	
Speakers: to be announced by NIDA	
Sponsored and organized by NIH/NIDA	
25th Scientific Conference of SNIP Begins	6:00 PM
Opening Night Reception & Early Career Investigator (ECI) Poster Session (1)	6:00 – 9:30 PM
Sponsored by the Early Career Investigator Award (ECITA) Committee	
***Posters W01 – W52** will stay up until Thursday's lunch break*	
DISC Networking Session	8:00 – 9:30 PM
Sponsored by the Diversity and Inclusion SNIP Committee (DISC)	

ThursdayApril 11, 2019

Breakfast	7:00 – 8:00 AM
President's Welcome	8:00 – 8:10 AM
Dr. Johnny He, Ph. D., Interim Chair & Professor	
Microbiology, Immunology & Genetics, University of North Texas Health Science Center,	
Fort Worth, TX, USA	
Presidential Symposium (1)	8:10 – 9:40 AM
Creativity in Research	
Co-Chairs: **Brian Wigdahl**, Ph.D., Drexel Univ. Philadelphia, PA	
Sanjay B. Maggirwar, Ph.D., Univ. Rochester, Rochester, NY	
Talk 1) **Sanjay Maggirwar**, Ph.D., Univ. of Rochester Medical Center, Rochester, NY	
"Creativity in Research: Avoiding the kiss of death"	
Talk 2) **Tariq Rana**, Ph.D., Univ. California San Diego, La Jolla, CA	
"Epigenetic and Epitranscriptomic Regulation of HIV infection"	
Talk 3) **Brandon K. Harvey**, Ph.D., National Institute on Drug Abuse, Baltimore, MD	
"Developing molecular tools: Sometimes your control is the interesting part of the experiment"	
Talk 4) **Mark Rizzo**, Ph.D., Univ. of Maryland School of Medicine, Baltimore, MD	
"Taking inspiration from other fields to tackle problems worth FRETting"	
Talk 5) **Carlos A. Paladini**, Ph.D., Univ. of Texas at San Antonio, San Antonio, TX	
"Hunting neurons deep in the brain: developing in vivo methods to suit your research questions"	
Break (posters available for viewing)	9:40 – 10:00 AM
Presidential Lecture	10:00 – 11:00 AM
Introduction: **Dr. Johnny He**, Ph. D., UNTHSC, Fort Worth, TX, USA	
Dr. Antonello Bonci, M.D., Scientific Director, NIDA, NIH (Currently on sabbatical)	
Synaptic Plasticity, Section Chief, NIDA IRP, NIH/NIDA, Baltimore, MD, USA	
"From optogenetics to a novel treatment against cocaine abuse"	
Symposium 2	11:00 – 12:15 PM
The Biology of Adenosine	
Chair: **Jonathan Geiger**, Ph.D., Univ. North Dakota, Grand Forks, ND	
Talk 1) **Anusha Mishra**, OHSU, Portland, OR,	
"The purinergic system in astrocyte biology and neurovascular coupling."	

Talk 2) **Munjal Acharya**, University of California Irvine, Irvine, CA,
"Targeting astrocytic adenosine kinase to remediate radiation-induced brain injury"

Talk 3) **Ursula Sandau**, OHSU, Portland, OR,
"Transient use of a systemic adenosine kinase inhibitor attenuates epilepsy development in mice"

Meet the Mentors Luncheon 12:15 – 2:00 PM
Sponsored by the Early Career Investigator Committee

Additional Council Meeting (Planning of next conference) 12:15 – 2:00 PM

Lunch Break / *Poster mounting **T01-T52** for Poster Session (2)* 12:15 – 2:00 PM

Young Investigators Session; ECITA Pre-& Post-Doc Symposium 2:00 – 3:45 PM
Co-Chairs: **Gurudutt Pendyala**, Ph.D., Univ. of Nebraska Med. Ctr., Omaha, NE,
Sylvia Fitting, Ph.D., University of North Carolina, Chapel Hill, NC, and
Dionna Williams, Ph.D., John Hopkins University, Baltimore, MD

Part I Predoctoral (*5 min presentations*)
Talk 1) **Michael Ohene-Nyako**, Rush University (Napier Lab)
"The blood-brain barrier is dysregulated in the hippocampus of Meth self-administering HIV-1 transgenic rats"

Talk 2) **Omalla Allan Olwenyi**, University of Nebraska Medical Center (Byrareddy Lab)
"Retinoic Acid (RA) enhances the Recovery of Replication Competent Virus from Latent SIV infected cells by activating $\alpha 4\beta 7$-expressing cells"

Talk 3) **Farah Shahjin**, University of Nebraska Medical Center (Yelamanchili Lab)
"Role of extracellular vesicles in HIV-1 and methamphetamine induced neurotoxicity"
Talk 4) **Hang Liu**, Temple University (Ho Lab)
"Changes of TLR3 Signaling Activation during Astroglial Differentiation"

Talk 5) **Jamie Marino**, Drexel University (Nonnemacher Lab)
"Effect of HIV-1 Tat-induced senescence on astrocytes and BMECs in a blood brain barrier model"

Talk 6) **Yuqing Gong**, University of Tennessee Health Sciences Center (Kumar Lab)
"Novel elvitegravir nanoformulation for drug delivery across the blood-brain barrier to achieve HIV-1 suppression in the CNS"

Q & A session part I

Part II Postdoctoral (*5 min presentations*)
Talk 1) **Silvia Torices**, Ph.D., University of Miami (Toborek Lab)
"HIV alters Occludin-Alix-Cav-1 interactions in human brain vascular pericytes"

Talk 2) **Hina Singh**, Ph.D., University of California at Riverside (Kaul Lab)
"Effect of IFNAR1 Deficiency on Neuronal and Astrocytic Cell Markers"

Talk 3) **Purnima Gupta**, Ph.D., Florida International University (Nair Lab)
"Efficacy studies of Magneto-electric nanoparticle bound Cas9/gRNA/Naltrexone to treat opioid addiction and neuroAIDS"

Talk 4) **Stephanie Matt**, Ph.D., Drexel University (Gaskill Lab)
"Role of dopamine-mediated conformational changes of CCR5 in HIV infection of macrophages"

Talk 5) **Andrew Atkins**, Ph.D., Drexel University (Wigdahl Lab)
"Validation of in silico predictions for gRNA specificity targeting HIV-1 proviral sequences using unbiased detection of CRISPR/Cas9 cleavage events"

Q & A session part II

Break 3:45 – 4:00 PM

NIH Workshop on Grant Writing for Trainees 4:00 – 5:00 PM
Participants:
Dr. Roger Sorenson, Ph.D., NIDA, NIH, MD USA
Dr. Woody Lin, M.D., Ph.D., NIDA, NIH, MD USA
Dr. Vasudev Rao, Ph.D., NIMH, NIH, MD USA
And **TBA**

Poster Session (2) 5:00 – 8:30 PM
Posters T01-T52
JNIP Editorial Board Meeting 8:00 – 10:00 PM

Friday April 12, 2019
Breakfast & Perkin Elmer presentation 7:00 – 8:00 AM

Symposium 3 8:00 – 9:30 AM
Glia, Toxins & Drugs of Abuse (in collaboration with the **Neurotoxicity Society**)
Chair: **Italo Mocchetti**, Ph. D., Georgetown University, Washington, DC

Talk 1) **Gilles J Guillemin**, Ph.D., Macquarie University, NSW, AUS,
"Glia, tryptophan metabolism and neurodegenerative diseases "
Talk 2) **Jean Harry**, Ph.D., NIH/NIEHS, RTP, NC,
"Modulatory ability of toxicants to shift regulatory processes of microglia and neuroinflammation"

Talk 3) **Julie K. Andersen**, Ph.D., The Buck Institute, Novato, CA,
"Senolytics as novel potential therapeutics for Alzheimer's and Parkinson's disease"

Talk 4) **Eliseo Eugenin**, Ph.D., University of Texas Medical Branch, Galveston, TX,
"Role of Glial cells in the pathogenesis of NeuroHIV"

Talk 5) **Kathleen Maguire-Zeiss**, Ph. D., Georgetown University Med Ctr, Washington, DC, "Aberrant proteins incite a complex glial inflammatory response"

2nd Adarsh Kumar Lecture – (DISC Lecture) 9:30 – 10:30 AM
Introduction: **Dr. Kelly Jordan-Scuitto**, Ph.D., Univ. of Pennsylvania, Philadelphia, PA

Dr. Susan Amara, Ph.D., Scientific Director, NIH/NIMH, Bethesda, MD
Title: TBA

Break 10:30 – 10:45 AM

Symposium 4 10:45 – 12:15PM
Blood-Brain Barrier Dysregulation Due to Drugs of Abuse and Pathogens
Co-chairs: **Michael Nonnemacher**, Ph.D., Drexel University, Philadelphia, PA
Pankaj Seth, Ph.D, FNASc, FIANS, National Brain Res. Ctr. (NBRC), Manesar, HR, India

Talk 1**) Jorge Alvarez**, Ph.D., Univ. of Pennsylvania, Philadelphia, PA,
"Migration/Reverse migration across the BBB as mechanisms of neuroinflammation"

Talk 2) **MaryPeace McRae**, Pharm.D., Ph.D., Virginia Commonwealth Univ, Richmond, VA, "Opiates and HIV-1 perturb the cellular/regional biodistribution of antiretrovirals in the brain by disrupting the blood-brain barrier and efflux transporter function"

Talk 3) **Michal Toborek**, Ph.D., University of Miami, Miami, FL,
"Pericytes and BBB in substance abuse"

Talk 4) **Richard Daneman**, Ph.D., University of California, San Diego, CA,
"Regulation of the Blood-Brain Barrier in Health and Disease"

Lunch (on your own) 12:15 PM
Exploration of Portland and surroundings Afternoon

Saturday April 13, 2019

Breakfast 7:00 – 8:00 AM

Symposium 5 8:00 – 9:40 AM
Addiction, Genetics and Neuroimmune Signaling
Chair: **Tamara Phillips**, Ph.D., VA Portland Health Care System, Research and Development Service, Portland, OR; Department of Behavioral Neuroscience, Portland Alcohol Research Center, and Methamphetamine Abuse Research Center, Oregon Health & Science University, Portland, OR

Talk 1) **Kathleen Grant**, Ph.D. Oregon National Primate Research Center, OHSU, OR, "Immunoreactive Effects of Long-Term Voluntary Ethanol Consumption in Non-Human Primates"

Talk 2) **Robert Hitzemann**, Ph.D. OHSU, Portland, OR,
"Genetic Findings from Mouse to Macaque to Man: Neuroimmune Function and Excessive Ethanol Consumption"

Talk 3) **William Hoffman**, M.D., Ph.D., VA Portland Health Care System, OHSU, Portland, OR, "The Relationship Between Interleukin-6 and Functional Connectivity in Methamphetamine Users"

Talk 4) **Jennifer Loftis**, Ph.D., VA Portland Health Care System, OHSU, Portland, OR,
"A Potential Neuroimmune-Based Treatment for Methamphetamine Addiction"

Break 9:40 – 10:00 AM

Bill Narayan Lecture 10:00 – 11:00 AM

Introduction: **Howard Fox**, M.D., Ph.D., Univ. of Nebraska Medical Center, Omaha, NE
Dr. Scott W. Wong, Ph.D., Vaccine and Gene Therapy Institute & Oregon Health & Science University, Portland, OR,
"Herpesvirus- and lentivirus-mediated neuroinflammation in the non-human primate"

Symposium 6 11:00 – 12:30 PM

Exosomes in Neuronal Infections and Drugs Abuse
Chair: **Santosh Kumar**, Ph. D., UTHSC, Memphis, TN
Co-chair**: TBA**

Talk 1) **Elena V Batrakova**, Ph.D., UNC, Chapel Hill, NC
"Macrophage-derived extracellular vesicles target inflamed brain and deliver therapeutic proteins for treatment of neurodegenerative disorders"

Talk 2) **Servio Ramirez**, PhD, Temple University, Philadelphia, PA,
"Extracellular vesicles: Mediators and biomarkers of pathology along CNS barriers"

Talk 3) **Santosh Kumar**, Ph. D., UTHSC, Memphis, TN,
"Circulating plasma exosomal cytochrome P450 and antioxidant enzymes and cellular processes"
Talk 4) **Mikin Patel**, Ph.D.cand., Vanderbilt Univ., Nashville, TN,
"Role of exosomes in synapse formation"
Open Business Meeting / Lunch 12:30 – 1:30 PM

Symposium 7 1:30 – 3:30 PM

SNIP Member Symposium: Neuropharmacology & Neuroimmunology
Co- Chairs: **Siddappa Byrareddy**, Ph.D., Univ. of Nebraska Med. Ctr., Omaha, NE
and **Roger Sorenson**, Ph.D., NIDA, NIH, Bethesda, MD, USA

Talk 1) **Alejandra Borjabad**, Ph.D., Icahn School of Medicine at Mount Sinai, New York, NY
"Epigenetic Therapy Approach in HIV-mediated Neurocognitive Impairment in Mice"

Talk 2) **Ernest Chivero**, Ph.D., Univ. of Nebraska Med Ctr., Omaha, NE
"Combinatorial effects of HIV Tat and cocaine-mediated activation of microglial NLRP3: Implications for NeuroHIV"

Talk 3) **Rick Meeker**, Ph.D., University of North Carolina, Chapel Hill, NC
"Neuroprotective and anti-inflammatory properties of the neurotrophin receptor ligand, LM11A-31, in animal models of HIV neuropathogenesis"

Talk 4) **Kimberly Williams**, Ph.D., University of Pennsylvania , Philadelphia , PA
"Suppression of Macrophage-Induced Neurotoxin Production via Estrogen Receptor Signaling"

Talk 5) **Ana Sanchez**, A.B., Ph.D., Uinv. California San Diego, La Jolla, CA
"Protease Inhibitors Effects on Vascular Smooth Muscle Cells"

Talk 6) **Jay McLaughlin**, Ph.D., University of Florida, Gainesville, FL
"Microglia-mediated neuroinflammation bidirectionally modulates morphine reward"

Talk 7) **Marina Aksenova**, Ph.D., University of South Carolina, Columbia, SC
"Inhibition of the DEAD Box RNA Helicase 3 prevents HIV-1 Tat- and cocaine-induced neurotoxicity by targeting microglia activation"

Talk 8) **Allison Andrews**, Ph.D., Temple University, Philadelphia, PA
"Characterization of differential monocyte subtype responses in cocaine-induced neuroinflammation"

Break 3:30 – 4:00 PM

Symposium 8 4:00 – 5:30 PM

Systemic Mechanisms of Neuroimmune Communication & Glymphatics

Co-Chair: **Michelle Erickson**, Ph.D., UW, Seattle, WA,
Kelly Jordan-Sciutto, Ph.D., UPenn, Philadelphia, PA

Talk 1) **William Banks**, Ph.D., UW, Seattle, WA,
"The Neuroimmune Axes of the Blood-brain Barrier"

Talk 2) **Jeffrey Iliff**, Ph.D., OHSU, Portland, OR,
"Glymphatic and meningeal lymphatic function in health and disease"

Talk 3) **Yisel Cantres-Rosario**, Ph.D., Univ. PR, San Juan, PR,
"Macrophage cathepsins as mediators of HAND"

Talk 4) **Michelle Erickson**, Ph.D., UW, Seattle, WA,
"Systemic mechanisms of neuroimmune communication from the lungs: implications for Alzheimer's disease and depression"

Break 5:30 – 7:00 PM

Banquet 7:00 – 10:00 PM

Hosted by the new SNIP president, **Dr. Sulie Chang**, Ph.D., Director of the Institute of NeuroImmune Pharmacology, Seton Hall University, South Orange, NJ
Announcements, Awards and Adjournment of Conference

Abstracts of the 25th SNIP Scientific Conference

Abstracts are ordered in three major groups: 1) speakers (S01-22 numbered alphabetically by last name of speaker but here sorted by event), 2) poster presenters Wednesday (W01-52) and 3) poster presenters Thursday (T01-52). Poster abstracts are ordered alphabetically by the last name of the first/presenting author.

1) Speakers (speaker abstracts available on 3/1/2019 are included)
Presidential Symposium (#1): Creativity in Research.

S16 Creativity in Research: Avoiding the kiss of death

Maggirwar, Sanjay B., Ph.D.[1]; [1]Department of Microbiology and Immunology, University of Rochester Medical Center, Rochester, NY, 14642 United States.

Improvements in the 'master' skill of creativity became an essential denominator for biomedical researchers in modern times, because their future careers are set against a backdrop of unusual dynamics that calls for growing requirement to demonstrate impact beyond publications with their research. Creativity can be learned. It just need bold steps - like welcoming 'whacky' thoughts, exploring unlikely sources, and following footsteps of creative leaders in other fields. While briefly discussing such examples, this talk is intended to provide preface to the accomplish speakers who will showcase their real-life experiences of employing organic ideas, technologies, avenues, and how it helped them in achieving important mile stones.

S19 Epigenetic and Epitranscriptomic Regulation of HIV infection

Rana, T, Ph.D.[1]; [1]University of California San Diego, School of Medicine, La Jolla, CA, 92093 United States.

Two interconnected themes will be discussed in this lecture. (1) We report the first genome-wide expression analysis of lncRNAs in HIV-1-infected primary monocyte-derived macrophages (MDM). We identified a lncRNA, which we named HIV-1 Enhanced LncRNA (HEAL), that is upregulated by HIV-1 infection of MDM, microglia, and T lymphocytes. Notably, HEAL knockdown and knockout mediated by RNAi and CRISPR-Cas9, respectively, prevent HIV-1 recrudescence in T cells and microglia upon cessation of azidothymidine treatment in vitro. These results suggest that silencing of HEAL or perturbation of the HEAL-ribonucleoprotein complex could provide a new strategy to eradicate viral reservoirs and effect a cure for HIV-1/AIDS. (2) N(6)-methyladenosine (m6A) is the most prevalent internal modification of eukaryotic mRNA. Very little is known of the function of m(6)A in the immune system or its role in host-pathogen interactions. We found that HIV infection affects viral and human RNAs by altering the topology and function of m6A, a modification affecting RNA structure and function. Our findings identify a new mechanism for the control of HIV-1 replication and their interactions with the host immune system. This discovery pioneered the new field of epitranscriptomics in virology and immunology and propelled advances in science and careers by pursuing the epitranscriptomic regulation in many other viruses including Influenza, ZIKA, Influenza, HCV, KSHV, and HBV.

Supported by NIH

Developing molecular tools: Sometimes your control is the interesting part of the experiment

Brandon K. Harvey, Ph.D., National Institute on Drug Abuse, Baltimore, MD

No abstract available at time of printing

S20 Taking inspiration from other fields to tackle problems worth FRETting

Rizzo, MA, Ph.D.[1], Markwardt, ML[1], Liang, J[1]; [1]Physiology, University of Maryland Sch of Medicine, Baltimore, MD, 21201 United States.

Fluorescent proteins form the backbone of many genetically-encoded protein and ion sensors. FRET-class sensors incorporate two proteins that sandwich a sensing motif to produce a ratiometric indicator. Recently, our lab has developed single-color FRET sensors that utilize two FRET partners of the same color. Three or more of these sensors can be used in the same specimen for multiplexed experimentation. Even so, the prominent mCherry red fluorescent protein has an unstable emission polarization, making it a poor choice for homotransfer biosensors. To improve mCherry's fluorescence properties, we took inspiration from 'Big Data' analytics methods and devised an in silico approach to generate a structural library using molecular modeling. Side-chain movements were correlated with changes in the chromophore orientation, and an aggressive mutagenesis approach led to chromophore stabilization in silico. The resulting protein, mVermilion, is twice as bright as mCherry and displays a superior polarization profile suitable for homotransfer biosensors.
Supported by NIH R01DK077140; R01 HL122827; R01MH111527

Developing molecular tools: Sometimes your control is the interesting part of the experiment

Carlos A. Paladini, Ph.D., Univ. of Texas at San Antonio, San Antonio, TX

No abstract available at time of printing

Presidential Lecture (Plenary Lecture#1)

From optogenetics to a novel treatment against cocaine abuse

Antonello Bonci, M.D., Scientific Director, NIDA, NIH (Currently on sabbatical)
Synaptic Plasticity, Section Chief, NIDA IRP, NIH/NIDA, Baltimore, MD, USA
No abstract available at time of printing

Symposium #2 The Biology of Adenosine.

The purinergic system in astrocyte biology and neurovascular coupling

Anusha Mishra, Ph.D., OHSU, Portland, OR
No abstract available at time of printing

Targeting astrocytic adenosine kinase to remediate radiation-induced brain injury

Munjal Acharya, Ph.D., University of California Irvine, Irvine, CA
No abstract available at time of printing

S21 Transient use of a systemic adenosine kinase inhibitor attenuates epilepsy development in mice

Sandau, US, Ph.D.[2], Yahya, M, BS[1], Bigej, R[1], Friedman, JL[1], Saleumvong, B, BS[1], Boison, D, Ph.D.[3]; [1]RS Dow Neurobiology Laboratories, Legacy Research Institute, Portland, OR, 97232 United States. [2]Department of Anesthesiology and Perioperative Medicine, Oregon Health and Science University, Portland, OR, 97239 United

States. [3]Department of Neurology, Oregon Health and Science University, Portland, OR, 97239 United States.

Over a third of all patients with epilepsy are refractory to treatment and there is an urgent need to develop new drugs that can prevent the development and progression of epilepsy. Increased expression of adenosine kinase (ADK) contributes to epileptogenesis and a target for therapeutic intervention. Here we tested the transient use of an ADK inhibitor administered during the latent phase of epileptogenesis to mitigate the development of epilepsy in the intrahippocampal kainic acid (KA) mouse model of temporal lobe epilepsy. KA injected mice were treated with the ADK inhibitor 5-iodotubercidin (5-ITU, 1.6 mg/kg bid i.p.) during the latent phase of epileptogenesis from day 3-8 after injury; the time period when gradual increases in hippocampal ADK expression begin to manifest. Hippocampal paroxysmal discharges (HPDs) were assessed at 6 and 9 wks post KA followed by epilepsy histopathology. 5-ITU significantly reduced the percent time in seizures by at least 80% in 56% of mice at 6 wks post KA. This reduction in seizure activity was maintained in 40% of 5-ITU treated mice at 9 wks. 5-ITU also suppressed granule cell dispersion, and prevented maladaptive ADK increases in these protected mice. Our results show that the transient use of a small molecule ADK inhibitor, given during the early stages of epileptogenesis, has antiepileptogenic disease modifying properties, which provides the rationale for further investigation into the development of a novel class of antiepileptogenic ADK inhibitors with increased efficacy for epilepsy prevention.

Supported by Citizens United for Research in Epilepsy (CURE); NIH (NS088024, NS065957)

Young Investigators Session; ECITA Pre-& Post-Doc Symposium (5 min Short Talks)

Please see poster abstracts W01-W52 labeled with *.

Symposium #3 Glia, Toxins & Drugs of Abuse (Sponsored by the Neurotoxicity Society)

S09 Using Kynurenine Pathway Metabolites as Biomarkers for Disease Prognosis, Progression and Response to Treatment

Guillemin, GJ[1]; [1]Faculty of Medicine and Health Sciences, Macquarie University, Mt Colah, NSW, 2109 Australia.

The kynurenine pathway (KP) of tryptophan metabolism is one of the major regulatory mechanisms of the immune response. Activation of the KP is implicated in the pathogenesis of a wide range of neuroinflammatory diseases. Several pro-inflammatory mediators can activate indoleamine 2,3 dioxygenase (IDO-1) one of the first and regulatory enzymes of the KP. A prolonged activation of the KP leads to production and accumulation of several neuroactive metabolites including the potent excitotoxin quinolinic acid (QUIN). Every brain cell type appears to express differently the KP enzymes and producing different KP metabolites. Over the last decade, together with our collaborators, we have shown that the KP is activated and QUIN level increases in most of the major neurodegenerative diseases (multiple sclerosis, Alzheimer's disease, amyotrophic lateral sclerosis, Parkinson's disease), neuropsychiatric disorders (suicidality, schizophrenia, autism) and cancers (glioblastomas, breast cancers). We demonstrated that some KP metabolites can be used as accurate biomarkers for the prognosis and the rate of progression of several diseases. Designing new, sensitive, accessible and low-cost tools is the critical next step to be able to use these markers in clinical, biological and hospital laboratories.

S10 Modulatory ability of toxicants to shift regulatory processes of microglia and neuroinflammation

Harry, GJ, Ph.D.[1]; [1]Neurotoxicology/National Toxicology Program Laboratory, National Institute of Environmental Health Sciences, RTP, NC, 27709 United States.

A concern for exposure to environmental agents lies with effects of low-level exposure and risk for disease or altered restorative capabilities. Inflammation-related effects may occur via induction of inflammatory response or modification of response upon insult. To examine the hypothesis that environmental toxicants could alter the normal function of immune cells, arsenic (iAs) was selected as an environmental contaminant and in cancer therapeutics. iAs induces DNA damage and production of reactive oxygen species; yet, the immunosuppressive properties may be of greater biological relevance. iAs (1uM;24hrs) ability to modify the inflammatory response of microglia was examined. Lipopolysaccharide (LPS)-induction of Tnfa, Il1a, Il1b, Inos, and Nlrp3 mRNA and protein was diminished. IL-4/IL-13 challenge showed a diminished induction of anti-inflammatory arginase 1 (Arg1) and Ym1. Inflammasome activation, as measured by ATP-induced IL-1beta release from LPS primed cells was diminished. mRNA levels for stress response or toll-like receptors and mitochondrial bioenergetics were not altered. Phagocytic capacity was decreased. Mice (42.5ppm arsenic drinking water, 6-wks (brain levels: 75ng/g) showed no elevation in Tnfa, Il1a, Il1b, Il6, and Inos, blunting of LPS-induction, and blunting of Arg1 and Ym1 in the hippocampus. Rather than anti-inflammatory, a diminished anti-inflammatory response and altered phagocytic capacity suggests a dysregulated response of microglia. An inability to mount a normal response to stimuli could affect multiple biological processes and outcome.

Supported by National Institute of Environmental Health Sciences/NIH

S01 The Potential Role of Brain Cell Senescence in Age-Related Neurodegenerative Diseases

Andersen, JK[1], Woods, G[1], Chinta, SJ[1], Rane, A[1], Chamoli, M[1]; [1]Andersen Lab, Buck Research Institute for Age Research, Novato, CA, 94945 United States.

In age-related neurodegenerative disorders including Parkinson's and Alzheimer's disease (PD and AD), neurodegeneration has historically been considered to occur primarily via apoptosis. Findings from our laboratory have recently suggested however that, in addition to apoptosis, an alternative cell fate may drive neurodegenerative phenotypes associated with these disorders–a process known as cellular senescence. Various types of stress within non-neuronal brain cells can drive them into this quiescent non-dividing state which prevents tumor formation, but also elicits the release of pro-inflammatory cytokines and other factors that may damage neighboring neurons. Data from the laboratory indeed suggests that ablation of senescent brain cells prevents neurodegeneration associated with various mouse models of age-related neurodegenerative disease and in human iPSC-derived culture models. Excitingly, this process may not only be confined to non-neuronal cells but may also occur within post-mitotic neurons in response to stress. Mechanisms involved in this process and their potential role in neurodegenerative diseases including PD and AD will be discussed.

S07 Glia Toxins & Drugs of Abuse

Eugenin, E, Ph.D.[1]; [1]Department of Neuroscience, Cell Biology and Anatomy, University of Texas Medical Branch, Galveston, TX, 77555 United States.

Viral persistence and chronic CNS damage are important features observed in the current HIV ART era. Currently, most HIV reservoirs within the CNS are microglia/macrophages as well as a small population of astrocytes. Our data indicates that gap junction, pannexin and ATP receptors amplify toxicity and apoptosis to neighboring uninfected cells. Our

current data identify the viral mRNA and proteins expressed in CNS viral reservoirs as well as the bystander mechanisms of damage with focus on single cell analysis (or combined analysis), laser capture, and subsequent proteomic, lipidomics, as well as metabolomics. Here we will discuss the unique aspects of HIV infection, persistence, bystander damage and potential treatments to eradicate and cure HIV.

This work was funded by The National Institute of Mental Health, grant MH096625, the National Institute of Neurological Disorders and Stroke, NS105584, and UTMB internal funding (to E.A.E)

S17 Aberrant proteins incite a complex glial inflammatory response

Maguire-Zeiss, KA, Ph.D.[1], Alshawi, S, BS[1], McCann, M, MS[1], Carey, S, BS[1], Sanchez, K, BS[1], Donnelly, A[1], Utley, N[1]; [1]Neuroscience, Georgetown University, Washington, DC, 20057 United States.

The regulation of the brain's internal environment, that is homeostasis, is largely regulated by astrocytes and microglia. Glial cells can recognize extracellular signals such as cytokines, chemokines, growth factors, and pathogenic proteins through receptor engagement transducing these signals into intracellular responses and subsequently releasing soluble effectors. Given this function it is not surprising that glial cells are critical for synaptic plasticity and the brain's various responses to aging and pathogens. In nearly all neurodegenerative disorders, astrocytes and glia play a role in neuroinflammation and disease progression. Relevant to Parkinson's disease (PD) and dementia with Lewy bodies (DLB), we have demonstrated that microglia increase expression and release of pro-inflammatory molecules (TNFα, IL1β) and matrix metalloproteinases in response to toll-like receptor 2 activation by oligomeric α-synuclein. We now extend this work and show that pretreatment of microglia with the mGluR5 agonist, CHPG, inhibits these proinflammatory responses as well as α-synuclein-mediated phagocytosis. We also demonstrate that monocyte chemoattractant protein-1 (MCP-1/CCL2) and complement component 3 (C3) are both upregulated in α-synuclein-treated astrocytes. These astrocyte-derived proteins in turn regulate microglial migration and microglial-directed synaptic pruning, which is the focus of our ongoing investigations. Overall, our work suggests that glial crosstalk is regulated by disease relevant proteins

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Adarsh Kumar Lecture – (DISC Lecture / Plenary Lecture #2)
TBA

Susan Amara, Ph.D., NIMH, Bethesda, MD

No abstract available at time of printing

Symposium #4 Blood-Brain Barrier Dysregulation Due to Drugs of Abuse and Pathogens

Migration/Reverse migration across the BBB as mechanisms of neuroinflammation

Jorge Alvarez, Ph.D., Univ. of Pennsylvania, Philadelphia, PA

No abstract available at time of printing

Opiates and HIV-1 perturb the cellular/regional biodistribution of antiretrovirals in the brain by disrupting the blood-brain barrier and efflux transporter function

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Poor antiretroviral (ARV) penetration likely contributes to HIV persistence within the brain. Opioid abuse, which can affect the severity of HIV-associated neuropathogenesis, may also affect BBB integrity and ARV brain concentrations. The effects of morphine and the HIV-1 regulatory protein, Tat, on BBB integrity and ARV brain concentrations were analyzed. Using a transgenic mice that conditionally expresses HIV-1 Tat1-86 in a GFAP-driven manner (Tat+), paracellular leakiness of fluorescent markers into the brain was examined. After 5 d of morphine (or placebo), dextrans of varying sizes (10, 40, and 70 kDa) were transcardially injected. Dextran brain tissue content was measured. In another set of mice, Tat and morphine effects on regional brain concentrations of a combination ARV regimen were quantified. Morphine, and to a lesser extent Tat, exposure resulted in increased dextran leakiness. Despite enhanced BBB breakdown, morphine decreased concentrations of select ARVs in a region-specific manner, leading to significantly lower brain levels of these drugs. P-glycoprotein, an efflux transporter for which some ARVs are substrates, was significantly increased by morphine exposure, which was consistent with the lower brain concentrations. One ARV drug, lamivudine, was unaffected by Tat or morphine exposure. These data suggest that Tat and morphine differentially alter BBB integrity. Also, morphine resulted in decreased brain concentrations of specific ARV drugs, which is potentially due to increased expression and function of P-glycoprotein.

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Pericytes and BBB in substance abuse

Michal Toborek, Ph.D., University of Miami, Miami, FL

No abstract available at time of printing

S05 Regulation of the Blood-Brain Barrier in Health and Disease

Daneman, Richard, Ph.D.[1]; [1]Pharmacology, UCSD, La Jolla, CA, 92093-0636 United States.

Vascular endothelial cells in the central nervous system (CNS) form a barrier that restricts the movement of molecules and ions between the blood and the brain. This blood-brain barrier (BBB) is crucial to ensure proper neuronal function and protect the CNS from injury and disease. Although the properties of the BBB are manifested in the endothelial cells, transplantation studies have found that the BBB is not intrinsic to the endothelial cells, but is induced by interactions with the neural cells. Here we use a genomic, genetic and molecular approach to elucidate the mechanisms that regulate the formation and function of the BBB. We have identified a critical role for pericytes in regulating the permeability of CNS vessels by inhibiting the properties that make endothelial cells leaky. Specifically, pericytes limit transcytosis through endothelial cells and the expression of leukocyte adhesion molecules in CNS endothelial cells, limiting CNS immune infiltration. Furthermore, we have developed methods to highly purify and gene profile endothelial cells from different tissues, and by comparing the transcriptional profile of brain endothelial cells with those purified from the liver and lung, we have generated a comprehensive resource of transcripts that are specific to the BBB forming endothelial cells of the brain. We have further examined the profile of CNS endothelial cells following injury and disease and have identified molecular mechanisms by which pericytes control BBB

formation, which are then disrupted during neurological disease leading to BBB dysfunction.

Supported by NIH/NINDS

Symposium #5 Addiction, Genetics and Neuroimmune Signaling
Addiction, Genetics and Neuroimmune Signaling – Symposium Description

Tamara Phillips, Ph.D., VA Portland Health Care System, Research and Development Service, Portland, OR; Department of Behavioral Neuroscience, Portland Alcohol Research Center, and Methamphetamine Abuse Research Center, Oregon Health & Science University, Portland, OR

Research performed by several groups indicates that high genetic risk for ethanol and methamphetamine intake and high levels of use are associated with significant differences in the expression of neuroimmune-related genes. Dr. Tamara Phillips, will chair this symposium, Addiction, Genetics and Neuroimmune Signaling or Function, featuring 4 speakers from the VA Portland Health Care System and Oregon Health & Science University, Portland, OR. Dr. Kathleen Grant will discuss the effect of long-term, heavy alcohol drinking on the transcriptional and functional profiles of circulating immune cells, which indicate a hyper-responsive state that can be traced to altered chromatin accessibility patterns. Dr. Robert Hitzemann will dissect transcriptional features across mouse, monkey and man for risk for, individual variation in, and consequences of excessive ethanol consumption, some of which implicate neuroimmune-related genes. Dr. William Hoffman will discuss resting-state functional connectivity and plasma IL-6 studies in adults diagnosed with methamphetamine dependence, compared to control subjects, which support the notion that inflammation and alterations in the mesocorticolimbic system contribute to MA use disorder. Finally, Dr. Jennifer Loftis will present data pertaining to a promising new treatment strategy to address methamphetamine-induced brain injury that utilizes an immunotherapy with recombinant T-cell receptor ligand constructs. This collection of presentations support the importance of neuroimmune signaling and function in drug addiction.

S08 Effect of Chronic Alcohol Drinking on Peripheral Immune System Responses

Grant, K A, Ph.D.[1], Messaoudi, I, Ph.D.[2]; [1]Oregon National Primate Research Center, Oregon Health & Science University, Beaverton, OR, 97003 United States. [2]Department of Molecular Biology & Biochemistry, University of California, Irvine, Irvine, CA, 92602 United States.

A rhesus monkey model of chronic oral alcohol self-administration was used to address the impact of heavy drinking (> 3.0 g/kg/day average intakes, a drink-equivalent of >12 drinks/day) on the transcriptional and functional profiles of circulating immune cells. Peripheral Blood Mononuclear Cells (PBMCs) were collected from male (n=8, 4 controls) and female (n=9, 3 controls) monkeys. Chronic heavy drinking for over 12 months resulted in robust transcriptional changes in both sexes. The differentially expressed genes originated primarily from dendritic cells and monocytes, again in both sexes. Heavy drinking also induced similar gene expression changes in spleen macrophages. Stimulation of PMBCs and splenic macrophages with LPS resulted in a hyperinflammatory response in heavy drinkers that was evident at both the protein and transcriptional level. Differentially expressed genes detected in monocytes or macrophages from heavy drinkers enriched to gene ontology terms associated with signaling and leukocyte activation pathways. Interestingly, heavy drinking resulted in changes in genes that encode histone and chromatin modifying enzymes. Additional analysis of chromatin accessibility indicated that heavy drinking results in increased accessibility in promoters and intergenic regions that regulate expression of several LPS responsive genes. Overall, chronic heavy alcohol consumption in male and rhesus female monkeys has a significant effect on PMBCs and spleen resident cells, resulting in a hyper-responsive state that can be traced to altered chromatin accessibility patterns.

Supported by National Institute of Alcohol Abuse and Alcoholism

S11 GENETIC FINDINGS FROM MOUSE TO MACAQUE TO MAN: NEUROIMMUNE FUNCTION AND EXCESSIVE ETHANOL CONSUMPTION

Hitzemann, RJ, Ph.D.[1], Lockwood, DR, Ph.D.[1], Darakjian, P, MS[1], Iancu, OD, Ph.D.[1], Searles, R, Ph.D.[2], Zheng, C, Ph.D.[3], McWeeney, S, Ph.D.[3], Grant, KA, Ph.D.[1]; [1]Department of Behavioral Neuroscience, Oregon Health and Science University, Portland, OR, 97239 United States. [2]Integrated Genomics, Oregon Health and Science University, Portland, OR, 97239 United States. [3]School of Medicine, Oregon Health and Science University, Portland, OR, 97239 United States.

The past 20 years have witnessed a revolution in how we examine the relationships between the brain transcriptome and behavior, including abnormal behaviors such as alcoholism and drug abuse. Beginning with cDNA- and micro-arrays and moving to newer technologies such as RNA-Seq, it is now possible to survey the entire genome for changes in gene expression, coexpression, and cosplicing networks. Research on alcohol abuse has long been a leader in this field; data have now been collected across multiple vertebrate species including mouse/rat, macaque and man. This approach has greatly enabled our ability to dissect the transcriptional features associated with the risk for, the individual variation in, and the consequences of excessive ethanol consumption. Some general trends have emerged, e.g. repeated observations that excessive ethanol consumption is associated with the remodeling of synaptic tethering and cell adhesion molecules (e.g. the protocadherins and the Dlg gene family), of the extracellular matrix (especially the ECM collagens) and of glutamate synaptic function (especially NMDA receptor function). Several groups have also observed that high ethanol preference is associated with significant differences in the expression of neuroimmune related genes. This has led to testing the effects of anti-inflammatory drugs to reduce ethanol consumption. The drugs being tested include PPAR agonists, PDE4 inhibitors, antibiotic microglial inhibitors and TBK/IKKe and IKKb inhibitors. Some of these drugs e.g. apremilast, are now being tested in the human laboratory setting.

Supported by AA13484, AA 10760, AA13510, and AA13641

S12 The relationship between interleukin-6 and functional connectivity in methamphetamine users

Hoffman, William, wfh, MD, Ph.D.[1], Kohno, Milky, MK, Ph.D.[1], Loftis, Jennifer, JM, Ph.D.[1], Huckans, M., MH, Ph.D.[1], Dennis, Laura, LD, BS[2], McCready, Holly, HM, BS[2]; [1]Psychiatry, Oregon Health & Science University, Portland, OR, 97239 United States. [2]Mental Health, VA Portland Health Care System, Portland, OR, 97239 United States.

Methamphetamine (MA) causes an increase in pro-inflammatory cytokines in animal models and in humans. Resulting activation of microglia and neuro-inflammation could, via effects on reward networks, mediate behavioral characteristics of addiction. We examined the relationship between interleukin-6 (IL-6) and corticolimbic and striatolimbic resting-state functional connectivity (RSFC). Thirty adults diagnosed with MA dependence and 20 control subjects underwent a resting-state functional magnetic resonance imaging (fMRI) scan and gave a blood sample for determination of plasma IL-6 levels. Seed-based RSFC analyses were performed to examine the interactive effect of group and IL-6 on ventral striatal and prefrontal connectivity. Within the MA group, IL-6 levels were positively related to

striatolimbic RSFC but negatively related to corticostriatal RSFC. Our findings with IL-6 support the idea that inflammation may at least partly mediate the link among MA use disorder, RSFC, and behavior, possibly via effects on mesolimbic and mesocortical dopaminergic systems.

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S15 A Potential Neuroimmune-Based Treatment for Methamphetamine Use Disorder

Loftis, J.M., Ph.D.[1], Vandenbark, A.A., Ph.D.[1], Huckans, M., Ph.D.[1]; [1]Research & Development, VA Portland Health Care System, Portland, OR, 97239 United States.

Methamphetamine is a central nervous system (CNS) psychostimulant with high abuse liability and immunotoxic effects on brain regions that regulate cognitive and psychiatric functions. Pharmacotherapeutic development for methamphetamine addiction has primarily focused on neurotransmitter systems; the results from clinical trials continue to be modest, with only marginally improved outcomes. New strategies are needed to help adults regain lost function, avoid relapse during early remission, and re-engage in work and relationships. Through a series of preclinical experiments we show that recombinant T-cell receptor ligand (RTL) constructs [partial major histocompatibility complex (pMHC) class II constructs linked to myelin oligodendroglial cell glycoprotein (MOG)-35-55 peptide (pDR2/MOG-35-55)] address important neuroimmune and behavioral effects of methamphetamine addiction. Partial MHC/neuroantigen peptide constructs bind to and downregulate expression of CD74 – the primary receptor for macrophage migration inhibitory factor (MIF) and a key inflammatory mediator which is increased following methamphetamine use. In rodent models we demonstrate that RTL immunotherapy: i) reduces methamphetamine seeking behavior, ii) improves learning and memory impairments induced by methamphetamine exposure, and iii) reduces the expression of inflammatory cytokines and chemokines in the MIF/CD74 signaling cascade [e.g., chemokine (C-C motif) ligand 2]. Immunotherapy with RTL constructs may offer a new treatment strategy for the treatment of methamphetamine-induced CNS injury.

Supported by National Institute on Drug Abuse (P50DA018165, R41DA039632) and Department of Veterans Affairs (2I01 BX000226, 1IK6BX004209)

Bill Narayan Lecture (Plenary #4)

S22 Herpesvirus- and lentivirus-mediated neuroinflammation in the non-human primate

Wong, S.W., Ph.D.[1,2]; [1]Vaccine and Gene Therapy Institute, Beaverton, OR 97006, Oregon Health & Science University, Portland, OR 97239, United States.

Japanese macaque encephalomyelitis (JME) is a naturally occurring spontaneous inflammatory demyelinating disease that affects the central nervous system (CNS) of Japanese macaques (JM) housed at the Oregon National Primate Research Center. Extensive characterization of JME reveals it possesses clinical, histopathological and immunopathological similarities with human inflammatory demyelinating disease, notably multiple sclerosis (MS). Here, we report that JME correlates with an active virus infection caused by Japanese macaque rhadinovirus (JMRV) in the CNS. JMRV infection was apparent by detection of abundant viral antigen in activated macrophages/microglia cells, and in brain microvascular endothelial cells. Serological studies suggest that chronic infection is associated with disease in greater than 90% of cases. More importantly, experimental JMRV infection of genetically susceptible animals yielded a condition resembling JME. Taken together, these studies suggest that JMRV is associated with JME-like disease and suggest that other factors in addition to virus infection are involved in progression of disease.

Symposium #6 Exosomes in Neuronal Infections and Drugs Abuse
S03 Macrophage-derived extracellular vesicles target inflamed brain and deliver therapeutic proteins for treatment of neurodegenerative disorders

Batrakova, E V, Ph.D.[1]; [1]Center for Nanotechnology in Drug Delivery , University of North Carolina at Chapel Hill, Chapel Hill, NC, 27599 United States.

The successful systemic delivery of therapeutic proteins to the brain have been hampered by poor penetration across the blood-brain barrier. The use of exosomes as 'natural nanoparticles' to deliver therapeutic proteins to the brain offers crucial advantages compared to other nanoparticulate drug delivery systems. Comprised of natural lipid bilayers with the abundance of adhesive proteins, exosomes readily interact with cellular membranes of target cells, and pass through biological barriers. We posit that exosomes secreted by monocytes and macrophages can provide an unprecedented opportunity to avoid entrapment in mononuclear phagocytes (as a part of a host immune system), and at the same time enhance delivery of incorporated therapeutic proteins to target cells ultimately increasing drug therapeutic efficacy. In light of this we developed a new exosome-based delivery system for various potent therapeutic proteins, including neurotrophic factors, antioxidants, and lysosomal enzymes. Therapeutic proteins were loaded into exosomes ex vivo using two approaches: (i) transfection of exosome-producing cells with therapeutic protein-encoding plasmid DNA; or (ii) incorporation the drug into naive exosomes released by macrophages. The second approach utilized various methods, including permeabilization of exosomal membranes with saponin, sonication, extrusion, or freeze-thaw cycles to achieve high loading efficiency. A reformation of exosomes upon sonication and extrusion, or permeabilization with saponin resulted in high loading efficiency, sustained release, and preservation of the therapeutic protein against proteases degradation. Exosomes were readily taken up by neuronal cells in vitro. A considerable amount of exosomes was detected in the inflamed brain in mice following intranasal administration. Exosomal formulations provided Overall, exosome-based formulations have a potential to be a versatile strategy to treat different devastating neurodegenerative disorders.

Supported by National Institutes of Health grant 1RO1 NS102412

Migration/Reverse migration across the BBB as mechanisms of neuroinflammation

Servio Ramirez, Ph.D., Temple University, Philadelphia, PA

No abstract available at time of printing

S14 CIRCULATING PLASMA EXOSOMAL CYTOCHROME P450 AND CELLULAR PROCESSES

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The presence and functional role of the major drug-metabolizing cytochrome P450 (CYP) enzymes in human plasma exosomes are unknown. In this study, we identified functional CYPs in human plasma exosomes, showed their ability to cross the blood-brain-barrier (BBB) and deliver to extrahepatic cells, and demonstrated their role in mediating xenobiotic-induced toxicity both in hepatic and extrahepatic cells. First, we isolated and characterized exosomes from plasma obtained from de-identified healthy individuals. We observed that the relative level of CYP enzymes, especially CYP2E1, in exosomes is

higher than in plasma, suggesting their specific packaging in exosomes. We also found that CYP2E1 is expressed relatively at higher level in plasma exosomes than exosomes derived from hepatic and monocytic cells. Further, we determined whether plasma exosomes can cross the BBB and deliver CYPs into myeloid cells using an in vitro BBB and in vivo animal models. The results showed a significant increase in the level of CYPs, especially CYP2E1, across the BBB in myeloid cells. Finally, we observed that the plasma exosomal CYP2E1 cargo is capable to induce ethanol- and acetaminophen-mediated toxicity in hepatocytes and monocytes. We also showed an increase in the level of plasma exosomal CYP2E1 in alcohol-drinking mice, and their effect on ethanol-induced toxicity in hepatic cells. This is the first evidence of the substantial expression and circulation of CYP2E1 in plasma exosomes and their crucial role in mediating xenobiotic-induced toxicity in distant cells including myeloid cells.

Supported by NIH AA-022063

S18 Role of exosomes in synapse formation

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Abstract not included for printing upon request of corresponding author.

Symposium #7 SNIP Member Symposium: Neuropharmacology & Neuroimmunology

Please see poster abstracts T01-T52 labeled with *.

Symposium #8 Systemic Mechanisms of Neuroimmune Communication & Glymphatics

S02 The Neuroimmune Axes of the Blood-brain Barrier

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The blood-brain barrier (BBB) first acts to separate circulating immune substances and cells from the central nervous system (CNS), thus making the CNS an immune-privileged area. However, the BBB then participates in several ways to reconnect in a regulated way the central nervous and immune systems. These mechanisms can be categorized as neuroimmune axes. This talk will explore these axes and give examples of each. The axes are: i) modulation of BBB interface functions by immune substances; ii) Transport, penetration, and uptake of neuroimmune substances; iii) Immune cell trafficking; iv) Immune secretions by the barrier cells; v) effects of immune substances on BBB integrity. These axes interact and BBB interface functions are further enriched because of the polarized nature of the BBB. These axes and their interactions are important in physiological function, are involved in disease states, and are important in drug development of brain diseases that involve the CNS.

Supported by VA/NIH

S13 Glymphatic and meningeal lymphatic function in health and disease

Iliff, J.J., Ph.D.[1]; [1]Department of Anesthesiology and Perioperative Medicine, Oregon Health & Science University, Portland, OR, 97239 United States.

Perivascular glymphatic exchange supports the clearance of solutes, including amyloid beta and tau, from the brain interstitium to the cerebrospinal fluid compartment. Meningeal lymphatic vessels support the clearance of CSF and CSF solutes via the cervical lymphatic drainage. Imaging experiments conducted in rodents suggests that these two functions are counter-regulated across the sleep-wake cycle, with more rapid glymphatic exchange during sleep than waking, and more rapid lymphatic efflux during waking. In addition, both glymphatic exchange and lymphatic clearance are slowed in the aging brain, suggesting that impairment of exchange into and out of the CSF compartment may be one feature of the aging brain that renders it vulnerable to protein mis-aggregation, as occurs in neurodegenerative diseases such as Alzheimer's and Parksonson's disease.

Supported by National Institutes of Health

S04 Macrophage cathepsins as mediators of HAND

Cantres-Rosario, YM, Ph.D.[1], Ortiz-Rodriguez, SC, BS[1], Santos, A, BS[1], Plaud , M, MS[1], Negron, K, BS[1], Cotto, B, BS[2], Langford, D, Ph.D.[2], Melendez, LM, Ph.D.[1]; [1]Microbiology and Medical Zoology, University of Puerto Rico, Medical Sciences Campus, San Juan, PR, 00935 United States. [2]Lewis Katz School of Medicine , Temple University, Philadelphia, PR, 19140 Puerto Rico.

HIV-associated neurocognitive disorders (HAND) prevail in infected individuals. HIV-infected macrophages cross the blood brain barrier, secreting viral and neurotoxic products, including cathepsin B (CATB). CATB interacts with serum amyloid p component (SAPC), inducing neuronal apoptosis by an unknown mechanism. CATB levels and activity are increased in the plasma of HIV-seropositive patients. In addition, CATB and SAPC are present in the brain of HAND patients, with higher expression in HIV-associated dementia (HAD) and Alzheimer's patients. Our goal is to identify the mechanisms of neuronal dysfunction triggered by CATB/SAPC complex secreted from HIV-infected macrophages. SK-N-SH and primary neurons were exposed to active recombinant histidine-tagged cathepsin B (His-CATB). His-CATB entry was tracked by intracellular flow cytometry, and neuronal dysfunction was verified by western blot. Neurons internalized His-CATB, an effect that was modulated by the levels of HIV infectivity and partially decreased by pre-treatment with anti-CATB antibody. Pre-treatment with CATB and SAPC antibodies decreased cleavage of caspase-3 and restored synaptophysin in neurons. Macrophage-derived extracellular vesicles (EVs) were tested for presence of CATB and SAPC. Both were secreted in EVs, with higher levels of CATB in HIV-infected EVs. In summary, macrophages secrete CATB/SAPC complex free and in EVs, which can be internalized by neurons, and can be targeted to decrease neuronal dysfunction. Therefore, CATB/SAPC complex represents a novel target for therapies against HAND.

Supported by NIH 1 SC1 GM113691-01, RCMI TPC U54MD007600, G12MD007579, R25GM061838, R01MH107340, P01DA037830, P30MH092177

S06 Systemic mechanisms of neuroimmune communication from the lungs: implications for Alzheimer's disease and depression

Erickson, MA, Ph.D.[1], Baumann, KK, BS[2], Liang, WS, BS[2], Thysell, J[1], Quaranta, D[1], Banks, WA, MD[1]; [1]Division of Gerontology and Geriatric Medicine, Department of Medicine/University of Washington, Seattle, WA, 98104 United States. [2]Research and Development, VA Puget Sound Healthcare System, Seattle, WA, 90108 United States.

The term 'blood-brain barrier' has historically been used to describe the highly specialized brain endothelium, which prevents the unregulated diffusion of substances into the CNS. We now appreciate that the blood-brain barrier also facilitates bidirectional communication between the CNS and

periphery, and thus is a regulatory interface. Recent works have revealed that blood-brain interface dysfunction occurs following respiratory tract infections, as well as exposure to inhaled environmental toxicants, suggesting that the blood-brain interface plays an important role in lung-brain communication. My symposium talk will discuss mechanisms by which ozone, a widespread toxicant in air pollution, can initiate integrated immune communication pathways between the lungs, liver, and brain. I will further outline possible mechanisms by which the acute phase protein, serum amyloid A, participates in the lung-liver-brain axis and contributes to aspects of ozone-induced CNS dysfunction, such as depression and Alzheimer's disease.

Supported by NIEHS/R21 ES029657-01

2) Posters – Wednesday

W01 Development of Nanodiamond-based Anti-HIV Drug Delivery Targeted to the Brain

Abshier, J, BS[1], Roy, U, Ph.D.[1], Drozd, V, Ph.D.[2], Mito, S, Ph.D.[3]; [1]Department of Health & Biomedical Sciences, The University of Texas Rio Grande Valley, Brownsville, TX, 78520 United States. [2]Center for the Study of Matter at Extreme Conditions, Florida International University, Miami, FL, 33174 United States. [3]Department of Chemistry, The University of Texas Rio Grande Valley, Edinburg, TX, 78539 United States.

Background - Combined antiretroviral therapy (cART) is considered to be widely acceptable therapy for Human Immunodeficiency Virus (HIV-1) infection. However, it does not cure the disease and ineffectively clears the virus from its reservoir organs, such as the brain. Since, most of the current cART drugs cannot cross the Blood Brain Barrier (BBB) which makes the brain a reservoir organ for HIV. There are many targeted drug design approach that has been investigated towards the brain. However, most of them are yet to establish in the clinics because of the associated toxicity to the neurons. The present study proposes the nanodiamond as an excipient for anti-HIV drug delivery to the brain. Being a nanosized carbon molecule with natural biocompatibility and non-toxic nature, it becomes more efficient drug carrier than other carbon-based materials. Considering its potential and importance, we have characterized unmodified and surface-modified (-COOH and -NH2) nanodiamond for its capacity to load the anti-HIV-1 drug efavirenz and observe it's biological stability in vitro. Methods - Nanodiamond was chemically characterized by different surface modification (-COOH and -NH2) in order to load optimum amount of anti-HIV drug efavirenz. Biologically characterized drugs were finally tested for its ability to cross Blood-Brain Barrier to deliver the drug to the brain and its therapeutic efficacy against HIV-1. Results - Unmodified nanodiamond conjugated drug formulation has significantly higher drug loading capacity than surface-modified nanodiamond with minimum toxicity.

Supported by University of Texas Rio Grande Valley

W02 Role of aurora kinase A and endolysosomes in Alzheimer's disease

Afghah, Zahra, MS[1], Jonathan D. Geiger, JD, Ph.D.[1], Xuesong Chen, XC, MD, Ph.D.[1]; [1]Biomedical Sciences, University of North Dakota, Grand Forks, ND, 58203 United States.

Alzheimer's disease (AD) is the leading cause of dementia worldwide in people older than 65 years of age and to date there are limited treatments for this neurodegenerative disease. Endolysosome de-acidification, which occurs in AD, has been linked to the pathogenesis of AD including increased amyloidogenesis and p-Tau accumulation. Thus, endolysosome acidification may represent a new therapeutic strategy against AD pathogenesis. Endolysosome acidification is maintained mainly by v-ATPases, v-ATPase activity is regulated by aurora kinase A (AURKA) an enzyme that is known to regulate such vital neuronal functions as microtubule organization, neuronal migration, and synaptic plasticity. Recently, AURKA activity was reported to be decreased in postmortem brain tissues of AD patients. Here, we tested the hypothesis that AURKA affects levels of Aβ and p-tau by altering endolysosome pH. We demonstrated that AURKA was expressed in primary cultured rat neurons and brains of C57BL/6J mature mice. Also, using immunofluorescent staining we showed that AURKA phosphorylation is significantly decreased in the hippocampus of female 3xTg-AD mice compared to C57BL/6J control. Furthermore, we found that activation of AURKA with anacardic acid resulted in endolysosomes acidification and decreased levels of Aβ, and that inhibiting AURKA using MLN8237 decreased AURKA phosphorylation and increased levels of Aβ. These results help elucidate additional mechanisms causing AD pathogenesis and may lead to the development of AURKA activators as novel therapeutic strategies to prevent and/or treat AD.

Supported by P30GM103329, R01MH100972, and R01MH105329

W03 Evaluating a broad-spectrum guide RNA to inactivate HIV-1 LTR-driven transcription

Allen, AGA, BS[1], Worell, SDW[1], Nwaozo, GCN[1], Sullivan, NTS, Ph.D.[1], Dampier, WD, Ph.D.[1], Homan, GH[1], Pirrone, VP, Ph.D.[1], Passic, SP, MS[1], Williams, JW[1], Nonnemacher, MRN, Ph.D.[1], Wigdahl, BW, Ph.D.[1]; [1]Department of Microbiology and Immunology, Drexel University College of Medicine, Philadelphia, PA, 19102 United States.

HIV-1 persistence is a major hurdle to a cure. Genomic editing with the CRISPR/Cas9 system holds promise to permanently excise or inactivate integrated provirus. Broad spectrum gRNAs were designed by isolating patient PBMCs and deep sequencing their LTRs. This resulted in the development of a broad-spectrum gRNA designated SMRT1. SMRT1 demonstrated knock-down of HIV-1 LTR-driven transcription in a transient transfection system. This resulted in a residual amount of LTR-driven transcription that was postulated to be due to a lack of delivery to all cells. To further elucidate the nature of the residual LTR-driven transcription, a novel dual florescence system was designed that used the NL4-3 HIV-1 molecular clone that also encoded GFP while the Cas9 expression system also encoded RFP and the anti-HIV-1 SMRT1 gRNA. Using these two plasmids it was shown that when the Cas9 system was active, there was a 98 percent reduction in GFP expression. Furthermore, when a VSV-G pseudotyped NL4-3 GFP was used and Cas9 (RFP) was delivered to cells, there was again an extensive reduction in GFP expression. SMRT1 was also able to significantly reduce viral replication resulting from the delivery of two different fully infectious HIV-1 isolates (IIIB and BaL). In the case of IIIB infection, SMRT1 was able to decrease viral replication almost down to basal levels. Moreover, SMRT1 was tested on a patient-derived isolate of HIV-1. These studies represent a step towards understanding the complex task of using CRISPR/Cas9 for HIV-1-targeted excision/inactivation therapy.

W04* Validation of in silico predictions for gRNA specificity targeting HIV-1 proviral sequences using unbiased detection of CRISPR/Cas9 cleavage events

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HIV-1 persistence has been attributed to integrated proviral DNA in latent reservoirs. Recent studies have removed the integrated HIV-1 provirus from individual cells using the CRISPR/Cas9 system. One of the challenges of a CRISPR/Cas9-based treatment has been evaluation of the specificity of the therapy across a wide range of patients. A successful excision-based therapy would require a set of gRNAs which recognize all viral quasispecies while minimizing off-target cleavage. In silico predictions indicate that gRNAs can be designed to fulfill both criteria. The current study validated these predictions. A high sensitivity DNA cleavage assay was used to measure the cleavage efficiency of gRNAs. Results showed a strong correlation between in silico predictions and in vitro performance for the set of broad-spectrum gRNAs tested. Validation of off-target efficiency predictions was performed by adapting the genome-wide unbiased identification of DSBs enabled by sequencing (GUIDE-Seq) method with next-generation sequencing. The GUIDE-Seq assay has been shown to identify the sequence locations of double-strand breaks (DSBs) in living cells including those generated by CRISPR/Cas9. This enabled high throughput quantification of off-target CRISPR/Cas9 activity with an increase in depth of coverage compared to the original GUIDE-Seq method in order to validate in silico predictions of gRNA specificity by detection of off-target cleavage events. Preliminary results indicate that the broad-spectrum gRNAs had high specificity with respect to HIV-1 with no detectable off-target hits.

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W05 PERK Haplotype Functions in HIV-Associated Neurocognitive Disorders

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The Unfolded Protein Response (UPR) is a signaling system which aims to re-establish protein homeostasis under conditions of ER stress. Several markers of UPR activation have been observed in the CNS of patients with HIV-Associated Neurocognitive Disorders (HAND), including phosphorylation of the kinase PERK and of its substrate eIF2α. Besides the adaptive function of the UPR, there is extensive evidence to suggest that severe or pro-longed stress can cause the cell to switch over to a maladaptive response, which could contribute to neurodegenerative phenotypes. PERK signaling has well established connections to such maladaptive responses, including prolonged translational attenuation leading to synaptic defects and induction of CHOP, a pro-apoptotic factor. PERK has two major haplotypes, A and B, differentiated by three single nucleotide polymorphisms, which encode amino acid changes in the resulting protein. Intriguingly, haplotype B is a risk factor for several neurodegenerative diseases. Furthermore, haplotype B has been demonstrated to result in different eIF2a phosphorylation levels than haplotype A, when subjected to the same ER stress. We thus hypothesize that the amino acid changes between PERK haplotypes cause haplotype B to respond more severely than A to the same ER stress in neurons, biasing it towards maladaptive signaling and disrupting normal neuronal function. Here we investigate the molecular mechanisms contributing to this enhanced response. These studies will inform the development of novel therapeutics targeting PERK modulation for HAND patients.

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W06* Development of exosomal extracellular vesicles (xEVs) as therapeutic nanocarriers targeting HIV neuropathology and opioid abuse.

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Human immunodeficiency virus (HIV) induces neurological dysfunction, ranging from dementia to mild neuropathy, including HIV-associated neurocognitive disorders (HAND). Approximately 21% of HIV-infected individuals use opioids, which has been shown to exacerbate HIV-induced neuropathology. Therapeutics are therefore needed to prevent/reduce the neuropathology in HIV-infected opiate abusers. Extracellular vesicles (EVs) which include apoptotic bodies, microvesicles, and exosomes are released by most healthy/virally-infected hematopoetic cells. During viral infection, exosomal EVs(xEVs) are critical for intercellular communication, modulation of anti-viral immune responses, and integral to cellular functions. We hypothesize that xEVs play an integral role in HIV-induced neuropathology, during HIV infection in opiate users. Here we investigated impact of morphine on HIV replication in astrocytes, xEV role in HIV neuropathology, and the efficacy of xEVs as therapeutic nanocarriers to delivering agents that block HIV neuropathology and opiate effects across the blood-brain-barrier(BBB). Characterization of xEVs via zeta potential and size distribution determined using Zetasizer, TEM imaging, and flow cytometry. Effect of gp120 on neuronal cell viability, in context of morphine exposure was investigated using neuronal cell line SH-SY5Y co-cultured with an in vitro BBB transwell model. BBB integrity and permeability assessed via TEER and a permeability assay. Nanoformulations consisting of magnetic nanoparticle (MNP) coupled to xEVs carrying T20(Fusin) and/or CTOP peptides to reduce gp120-induced neuropathy and opioid-mediated effects on neurons were synthesized. These magneto-xEV-coupled nano-particles (mxEVs) nanoformulations were characterized, transport efficiency determined via ferrous assay, and loading capacity ascertained. Results show increased xEVs were transported across BBB when coupled to MNPs. SH-SY5Y viability was not significantly modulated by mxEVs as measured by XTT assays. Preliminary efficacy studies using SH-SY5Y co-cultured with BBB model showed that mxEV-T20-fusion peptides altered HIV gp120-mediated neurotoxicity. In summary, these preliminary findings support xEVs as neuroimmunomodulators which may exacerbate HIV-induced neuropathology, in the context of opiate abuse, and potential therapeutic nanocarriers.

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W07* HIV-Tat Protein Modulates Cognitive Performance through Induction of Hyperglutamatergic Signaling in Brain

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While combinatorial antiretroviral therapy (cART) has reduced the severity of HIV-associated pathology like HIV Associated Neurocognitive Disorder (HAND), the prevalence of HAND in HIV-positive patients has grown paradoxically worse. We hypothesize that persistent viral protein production in the brain is sufficient to mediate HAND symptomology. We correlated the effects of CNS-expression of HIV Tat

protein on glutamatergic signaling in brain circuits of the prefrontal cortex and hippocampus to Tat's effect on various cognitive domains in behavioral models of mood and cognition. Exposure to HIV Tat protein facilitated layer 2/3 pyramidal neuron firing (128 Â± 22%) in the mPFC and enhanced attentional set shifting. In constrast, HIV Tat protein delayed without altering average CA1 pyramidal neuron firing (94 Â± 14%; delay rectified after 8 current steps; 25 pA per 1 current step) and impaired spatial learning and memory performance. Assays of behavioral disinhibition and depression demonstrated anxiogenic and depressive-like effects respectively. Treatment with the NR2B antagonist ifenprodil or the glutaminase inhibitor JHU-083 prevented Tat-induced depression-like behavior, but was without effect on deficits of learning and memory on novel object recognition. In summary, acute exposure to HIV Tat protein seems sufficient to mediate many but not all aspects of HAND, at least partially through the dysregulation of the glutamatergic system in a brain region-specific manner that may benefit from therapeutic intervention.

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W08 Pharmacologic Inhibition of Cell-Associated and Exosomal P38 Mitogen-Activated Protein Kinase from Astrocyte Cells Exposed to Tumor Necrosis Factor-alpha

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It is known that p38 Mitogen-Activated Protein Kinase (p38MAPK) signaling pathway plays a crucial role in the pathogenesis of neurodegenerative diseases (ND). However, the development of novel therapeutic strategies targeting p38MAPK remains a major challenge but a promising therapeutic method. In this study we investigated if a TNF-alpha receptor 1 (TNFR1) antagonist, R7050, regulates the phosphorylation levels of p38MAPK (phospho-p38MAPK) in astrocyte cells and its release in exosomes in response to the proinflammatory cytokine, TNF-alpha (TNF). Human astrocytoma cells (U-87MG;5x10^5) were cultured in presence of TNF (10pg/mL) or TNF plus R7050 (10nM) for 24h. The levels of phospho-p38MAPK were measured in cells and released exosomes by flow cytometry using a specific anti-phospho-p38 antibody. Our results indicated that: (1)Phospho-p38MAPK levels decreased significantly(p

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W09 Mechanism of HIV CNS Damage Mediated by Lipids

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A critical comorbidity of HIV infection is the development of HIV-associated neurocognitive impairment (HAND) in HIV-infected individuals even in the antiretroviral therapies (ARTs) era. Although ARTs are effective treatments to block the systemic viral replication, they cannot block HIV protein synthesis. The mechanism of HAND is based on multifactorial process correlated to an early transmigration of HIV infected cells into the brain, infection of microglia/macrophages and a small population of astrocytes, as well as bystander damage of uninfected cells by host and viral mediators. HIV-infected astrocytes can induce bystander damage into neurons, astrocytes and endothelial cells via Connexin 43 (Cx43) containing gap junctions (GJs) and unopposed hemichannels (uHCs). The latter allow the secretion in the extracellular space of inflammatory signals such as Prostaglandin E2 (PGE2), a lipid compound taking part of the arachidonic acid related pathways. Notably, our results indicate that PGE2 is elevated in the sera of HIV-infected patients despite effective ARTs. Additionally, our Mass Spectrometry Imaging (MSI) preliminary data suggest that sulfatide is a key detector marker of the central nervous system (CNS) damage. Sulfatide is a ceramide-related pathway metabolite and its alteration is related with neurocognitive disorders such as Alzheimer's disease and Parkinson's disease. Thus, we propose that lipid dysregulation in HAND is mediated by HIV proteins produced by HIV-infected astrocyte and it is a potential marker of cognitive disorders.

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W10 CRISPR-Cas9 mediated disruption of ALCAM gene in uninfected and HIV+ myeloid cells inhibits their adhesion to endothelial cells

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Viral spread and seeding of different anatomical compartments within the body depends on cell-to-cell transmission and the ability of infected cells to disseminate by intra- and extravasation. ALCAM is a junctional protein over-expressed on activated T cells, monocytes, dendritic cells and also endothelium. Recent studies show increased levels of ALCAM on CD14+/CD16+ monocytes derived from HIV+ patients and a critical role in promoting transmigration of these cells across the blood brain barrier. Here we designed a pair of CRISPR guide RNAs targeting exon 1 of the human ALCAM gene. Successful cleavage of the target sites leads to deletion of the segment of DNA spanning the ALCAM start codon/signal peptide and to complete block of ALCAM expression. Using lentiviral delivery we created several single cell ALCAM knockout clones in pro-monocytic U937, and their latently infected with HIV-1 equivalent: U1 cells. CRISPR-Cas9 cleavage of ALCAM gene was verified by PCR based excision assay and Sanger sequencing of truncated, double cleaved/end-joined amplicons. The lack of ALCAM expression was confirmed by RT-qPCRs and flow cytometry. Next control and knockout cells were tested in adhesion assay using WT, and newly created ALCAM-/- cerebral endothelial cells, hCMEC/D3. All ALCAM knockout myeloid cell clones showed reduced adhesion to WT endothelial cells but not to ALCAM -/- ones. The approach presented here provides a new, gene editing based therapeutic avenue to treat any neuroinflammatory disorder with trafficking of immune cells component.

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W11 HIV Tat protein induced TGF-beta suppresses CFTR biogenesis and activity by microRNA mediated gene silencing

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The advent of combination antiretroviral therapy (cART) has led to a dramatic decline in morbidity and mortality from HIV/AIDS. HIV infected subjects are still six times more likely to contract pneumonia compared to non-infected age matched controls. Mortality, following an episode of bacterial pneumonia was also four times higher in HIV infected subjects compared to non-infected controls. Thus, pneumonia can also contribute to HIV progression. Understanding the pathophysiological mechanisms that lead

to microbial colonization of the airways in HIV infected patients is therefore important to public health. HIV Tat and CS can suppress CFTR function, which is a critical determinant of ASL depth and this can lead to depressed MCC and consequent microbial colonization. Our study showed that HIV-infected cells in the airway namely alveolar macrophages, other immune cells or bronchial epithelial cells can serve as reservoirs and a source of HIV proteins like Tat which upregulates TGF-beta 1 mRNA in primary human bronchial epithelial cells with a corresponding decrease in CFTR mRNA. Chromatin immunoprecipitation with RNA Polymerase II demonstrated that transcription from the CFTR promoter is unaffected and blocking the miRNA processing pathway with Aurin Tricarboxylic acid (ATA) restore CFTR inhibition caused by TGF- beta. Our data showed that HIV infection suppress CFTR mRNA and function and blocking TGF-beta signaling rescues this. Together this posits a strong role for (TGF-beta induced) miRNA in Tat mediated CFTR gene silencing.

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W12 Astrocyte HIV-1 Proviral Reservoirs in HAND

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Even though astrocytes are restrictively infected with HIV-1, they are capable of producing neurotoxic viral proteins. This can significantly aggravate HAND pathogenesis. Thus, there is a great need to identify latently infected astrocytes and develop strategies to target this specific population. We hypothesize that HIV-1 proviral reservoirs alter astrocyte function in conjunction with unique gene expression patterns that could serve as biomarkers and facilitate targeted therapy. Red/Green-HIV-1 (R/G-HIV-1) was used to visualize viral promoter (LTR) activity in primary human astrocytes. Astrocytes with active (R+/G+) and silent (R+/G-) LTRs were enriched using FACS. Alu-gag PCR confirmed the presence of integrated R/G-HIV-1 provirus in transduced astrocytes. Astrocytes with silent promoter activity were devoid of late viral proteins such as p24, indicating a functionally silent HIV-1 LTR. However, interluekin-1β (IL-1β) and Vorinostat, both reactivated silent HIV-1 LTR in R/G-HIV-1-infected astrocytes. Astrocytes with silent (R+/G-) and active (R+/G+) LTRs have significantly impaired glutamate clearance ability and cell proliferation compared to exposed uninfected (R-/G-) cells. Our data suggest that harboring HIV-1 provirus with either active or silent viral promoters interfered with astrocyte function and growth. Hence, we propose that identifying biomarkers for astrocytes harboring HIV provirus, and therapeutic gene editing to eliminate proviral gene expression, will improve physiological function compared to HIV-1 infected cells.

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W13 Interactions between nicotine and antiretrovirals in an in vitro model of HIV-induced neurotoxicity.

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Even though the mortality of HIV has decreased due to antiretrovirals (ARVs), HIV-associated neurocognitive disorders (HAND) remain prevalent, affecting 50% of HIV+ patients. HAND is a spectrum of cognitive, memory, and motor dysfunction that can present with mood and addictive disorders. Specifically, 50%â€“70% of HIV patients abuse nicotine, emphasizing the importance of studying the effects of nicotine on ARV-mediated suppression of HIV replication and associated neurotoxicity. Previous in vitro studies indicate that HIV replication in macrophages and microglia can be altered in the presence of nicotine or cigarette smoke extract. However, these studies were not conducted in the presence of ARVs, which were shown to cause direct toxicity to neurons. Importantly, indirect ARV-mediated neurotoxicity via their effect on macrophages remains unknown. We hypothesize that nicotine and certain ARVs interact to alter HIV replication in macrophages and subsequent cytokine and/or neurotoxin release. Specifically, we hypothesize that nicotine increases HIV replication and ARV-associated indirect neurotoxicity while decreasing the effectiveness of ARVs. Mock and HIV-infected monocyte-derived macrophages (HIV-MDMs) were treated with nicotine and/or the ARV lopinavir simultaneously. Supernatants were analyzed to assess HIV replication and HIV-MDM-induced neurotoxicity on three week old neuronal cultures. Understanding the interaction of nicotine and ARVs will aid in development of therapeutics that alleviate neurotoxicity and reduce the rate of substance abuse in people living with HIV.

Supported by MH109382

W14* Novel elvitegravir nanoformulation for drug delivery across the blood-brain barrier to achieve HIV-1 suppression in the CNS

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Over the last two decades, the use of antiretroviral therapy (ART) has remarkably decreased the morbidity associated with HIV-1 infection. However, the prevalence of HIV-1-associated neurocognitive disorders (HAND) is still increasing. The blood-brain barrier (BBB) is the major impediment for penetration of antiretroviral drugs, causing therapeutics to reach only suboptimal level after systemic administration. Therefore, conventional antiretroviral drug regimens may not be sufficient to improve the treatment outcome of HAND. We have developed a poloxamer-PLGA nanoformulation of elvitegravir (EVG), a commonly-used antiretroviral drug. This EVG nanoformulation showed improved intracellular uptake and viral suppression in HIV-1-infected macrophages. Recently, we assessed the BBB penetration of this EVG nanoformulation. Compared with EVG native drug, our EVG nanoformulation demonstrated an improved BBB penetration in both in vitro BBB model and in vivo mouse model. Most importantly, this EVG nanoformulation was able to show the enhanced HIV-1 suppression in HIV-1-infected human monocytic-derived macrophages and monocytic-derived microglia after crossing the BBB without changing the BBB integrity. Overall, this is an innovative and novel treatment strategy that has a potential for therapeutic interventions in reducing HAND.

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W15* Efficacy studies of Magneto-electric nanoparticle bound Cas9/gRNA/Naltrexone to treat opioid addiction and neuroAIDS

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Magneto-electric nanoparticles (MENPs) are one of the emerging multifunctional materials in the field of nanomedicine. The combination of MENPs to traditional drug delivery system, increases the efficiency of the treatment for brain disorders, thus, enabling tracking of drug carrier and monitoring their effective accumulation in the targeted tissues. RNA directed gene editing strategy using specific Cas9/gRNA has been used recently to eliminate integrated HIV genome. Dugs of abuse such as opioids are

frequently used by HIV infected subject. In this study, we developed a magneto-electric liposomes (MELs), a nanoformulation comprising of MENPs, Cas9/gRNA and Naltrexone hydrochloride (NTX). This MELs will serve as an effective therapeutic cargo to deliver Cas9/gRNA for the recognition and complete eradication of the HIV reservoir in the brain. In addition to this, NTX, an opioid antagonist would subside the risk of acquiring HIV infection. The synthesized MENPs exhibited superparamagnetic property. Further, these MENP bound Cas9/gRNA and NTX were loaded into the liposomes using a pH gradient and thin film hydration method. The entrapment efficiency of NTX and MENP was around 41% and 15%, respectively with a hydrodynamic size of 150-180 nm and a good polydispersity index. Importantly, these MELs were stable, biocompatible, safe and non-toxic to primary CNS cells. Also, these MELs achieved enhanced transmigration across an in vitro blood brain barrier model via magnteic targeting. This MENP bound Cas9/gRNA significantly reduced the HIV-LTR expression in HIV latent microglia ce

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W16 Morphine-induced iron release from endolysosomes is sufficient to cause reactive oxygen species production and mitochondrial dysfunction

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Mitochondria dysfunction and reactive oxygen species (ROS) production play important roles in healthy aging and in age-related neurodegenerative diseases including Alzheimer's disease, Parkinson's disease, and HIV-associated neurocognitive disorders (HAND). Because ferrous iron is a key factor for the generation of ROS via the Fenton reaction, and because intracellular and intra-mitochondrial iron originates from the endocytosis of ferric iron bound to transferrin, we explored the extent to which and mechanisms by which iron released from endolysosomes is an early and upstream event of mitochondria dysfunction, ROS production, and cell death. We demonstrated, in U87 glioblastoma cells, that morphine-induced endolysosome de-acidification increased the release of iron from endolysosomes and this resulted in decreased iron levels in endolysosomes, increased levels of iron in cytosol, and increased levels of iron in mitochondria. Iron released from endolysosomes into the cytosol by morphine resulted in increased ROS levels in both the cytosol and mitochondria and those increased levels of ROS were blocked by chelating endolysosome iron with deferoxamine. Mechanistically, we demonstrated that endolysosome-resident two-pore channels were involved in iron release from endolysosomes upon de-acidification, and that mitochondrial permeability transition pores were involved in iron uptake into mitochondria. Our findings suggest that endolysosome de-acidification and iron release from endolysosomes play important and early roles in mitochondrial dysfunction including ROS production.

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W17 The effects of retinoic acid and drugs of abuse on enteric glial cells

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The enteric nervous system is a complex system of neural plexuses, microglia-like macrophages, and enteric glia (EGCs). EGCs are similar to astrocytes and maintain intestinal barrier integrity, modulate immune cell response, and mediate the gut-brain axis (GBA). Because of the role the GBA plays in CNS health and the gut's importance in HIV pathogenesis, we aimed to understand the role of EGC's in HIV and drugs of abuse. We treated a rat EGC cells with physiologically relevant levels of retinoic acid (RA), Tat, Lipopolysaccharide (LPS), morphine, methamphetamine, and cocaine in relevant control/combination for 16 hours. We harvested the cells, extracted mRNA, and performed qPCR to measure gene expression for IL-1β, TNF-α, and TGF-β. Exposure to RA enhanced IL-1β and TNF-α expression by 5x and 29x respectively with Tat and LPS treatment. RA also allowed TGF-β to return to baseline level after a 4.5x increase with Tat and LPS. This inflammatory response potentially allows for increased T cell survival and a decreased risk of intestinal fibrosis. Our early findings also indicate that drugs of abuse alone do not cause inflammation, but in the presence of Tat and LPS, morphine, methamphetamine, and cocaine, IL-1β expression increased by approximately 5x, 2x, 6x, and TNF-α expression by 100x, 150x, and 90x, respectively. Additionally, each of these drugs increased TGF-β expression by 10x, 18x, and 9x, indicating that drug abusers may be more susceptible to accelerated disease pathogenesis when exposed to the virus. Future studies will be performed in primary HIV infected cells.

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W18 PET imaging of 11C-UCB-J and 11C-JHU75528 ligands indicate synaptic decline and increased levels of CB1R expression in HIV-1 Tat transgenic mice

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HIV-1 associated neurocognitive disorders (HAND) affect 50% of HIV+ patients. Although combined anti-retroviral therapies (cART) have worked to decrease the progression of HIV-1 towards AIDS and death, its limited penetration into the CNS is inefficient to treat HAND. Therefore, it is necessary to research and develop neuroprotective therapies that target the CNS. HAND is characterized by severe and minor cognitive decline linked to synaptodendritic neuronal injury. Using a Tat transgenic mouse model, previous work in our lab has demonstrated that CNS expression of HIV-1 Tat protein alone is able to recapitulate the neurodegenerative effects of HAND. In the present study we analyzed ubiquitous synaptic densities in the Tat transgenic mouse model via positron-emission tomography (PET) imaging using the synaptic vesicle glycoprotein 2A (SV2A) selective radiotracer, 11C-UCB-J. Preliminary data indicate a downregulation of SV2A (~8%) in the cerebrum of transgenic Tat(+) mice compared to Tat(-) controls. Interestingly, immunohistochemistry and neuroimaging of PET with the cannabinoid 1 receptor (CB1R) radiotracer, 11C-JHU75528, demonstrated a significant upregulation of CB1R expression (~26%) in the same region. These findings suggest there is a decrease in synaptic integrity due to Tat, which the

endocannabinoid system can be potentially compensating for with the upregulation of CB1R expression. Further manipulation of the endocannabinoid system may be critical to understanding its therapeutic potential for HAND.

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W19 Histaminergic Dysregulation in the HIV-1 Transgenic Rat: Implications for HIV- Associated Neurocognitive Disorder

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Histamine in the central nervous system has been suggested to play a role in neurocognitive impairments. Histamine release and presence of HIV-1 viral proteins are positively correlated, and HIV-1 patients exhibit increased spontaneous histamine release. We hypothesized that a dysregulated histaminergic system contributes to HIV-1 associated neurocognitive disorders. Using HIV-1 transgenic rats (n=7 male and 8 female) and F344 control rats (n=11 male and 9 female), fast scan cyclic voltammetry was performed. A stimulating electrode was implanted into the medial forebrain bundle and a carbon fiber recording microelectrode was placed in the posterior hypothalamus. The HIV-1 Tg rats showed increased histamine release relative to controls. In order to assess the source of the increased histamine, immunohistochemistry was performed on HIV-1 Tg (n=9 male and 5 female) and F344 rat (n=9 male and 5 female) brains to identify and quantify histaminergic cell bodies in the tuberomammillary nucleus of the posterior hypothalamus. There was no significant effect of histaminergic neuron numbers in HIV-1 Tg rats; however, there was a significant effect of sex on histamine neurons (p

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W20 HIV-1 gp120-induced endolysosome deacidification causes endolysosome iron release and increased levels of reactive oxygen species

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HIV-associated neurocognitive disorders (HAND) affects about 50% of HIV-positive individuals despite viral suppression achieved using antiretroviral therapies (ART). Pathologically, brain tissue from HAND patients have shown morphological changes in organelles such as endolysosomes and mitochondria. Mechanistically, HIV-1 proteins have been implicated in HAND pathogenesis. HIV-positive individuals show elevated levels of iron in serum, increased levels of reactive oxygen species (ROS), and iron chelators have been suggested as adjuvant therapies to ART. Furthermore, HIV-1 gp120 has been found to increase levels of ROS in neurons and other brain cells. Here, we tested the hypothesis that HIV-1 gp120-induced de-acidification of endolysosomes leads to an efflux of iron from endolysosomes and a subsequent increase in levels of ROS in cytoplasm and mitochondria of neural cells. We used U87MG glioblastoma cells and time-lapse confocal microscopy to measure gp120-induced changes in endolysosome pH, endolysosome iron, cytosolic and mitochondrial iron, and cytosolic and mitochondrial ROS. HIV-1 gp120 de-acidified endolysosomes, reduced endolysosome iron levels, increased levels of cytosolic and mitochondrial iron, and increased levels of cytosolic and mitochondrial ROS. These effects were all attenuated significantly by the iron chelator deferoxamine that only enters cells via endocytosis. These results suggest that cellular and subcellular effects of HIV-1 gp120 are downstream of its ability to de-acidify endolysosomes and increase the release of iron from endolysosomes.

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W21 MJN110 Therapeutic Potential Against HIV-1 Tat-induced Neurotoxicity In Vitro

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The advent of combined antiretroviral therapy (cART) has greatly increased expected longevity in persons living with HIV-1 (PLWH), but therapeutics targeting residual viral proteins are largely unestablished. Transactivator of transcription (Tat) is among the first viral proteins to enter the central nervous system upon initial infection, and is poorly suppressed by cART. Previous work has demonstrated both the role of Tat in development of neurocognitive deficits which contribute to phenotypes of HIV-associated neurocognitive disorder, and the protective role of the endocannabinoid system in ameliorating neural dysfunction. Thus, the present study aimed to investigate the potential utility of blocking enzymatic breakdown of endocannabinoid ligand 2-arachidonoylglycerol (2-AG) with monoacylglycerol lipase inhibitor MJN110 in Tat-treated prefrontal cortex neuron cultures. Tat exposure significantly increased neuron excitability induced by 100 nM - 50 μM glutamate application in live-cell microscopic recordings of intracellular calcium ([Ca2+]i) activity, an effect which was significantly reduced in cultures treated with 500 nM and 1 μM MJN110. The observed effect was time- and concentration-dependent, such that longer MJN110 exposure at higher concentrations more effectively protected against excitotoxicity. Further investigations will examine behavioral outcomes of MJN110 treatment against Tat-induced deficits in vivo to ultimately identify therapeutic targets for mitigation of HAND symptoms in PLWH.

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W22* Changes of TLR3 Signaling Activation during Astroglial Differentiation

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Toll-like receptor 3 (TLR3)-mediated antiviral signaling is critical to the innate immunity of central neural system (CNS). However, as a major participant of brain innate immunity, astroglial TLR3 expression during

differentiation remains unclear. We here use an astroglial differentiation model from human neural stem cells (H9-derived; hNSC/H9) to investigate the changes of TLR3 expression and activation during this period. Undifferentiated hNSC/H9 cells expressed little endosomal TLR3 and were inert to directly naked PolyI:C treatment in terms of less IFN-λ expression. With the conduct of hNSC/H9 differentiation, the distribution of TLR3 ranges from the intracellularly to the astroglial cell surface in most cells, and the total expression of TLR3 increased concordantly with the steadily increased IFN-λ induction by PolyI:C treatment. In addition, naked PolyI:C treatment was able to upregulate IFN-λ expression in primary astrocytes whereas intracellularly delivered PolyI:C transfection (lyo-PolyI:C) induced much stronger IFN-λ expression. Moreover, co-culture experiments showed that primary astrocytes with lyo-PolyI:C treatment could inhibit HIV-1 replication in macrophages. This inhibition could be partially reversed by pretreatment of macrophages with neutralization antibody to IFN-λ receptor. In summary, our data for the first time demonstrated the changes of spatiotemporal distribution and activation of TLR3 during the differentiation of NSC to astrocytes and implicated an active regulatory bystander role of astrocytes against viral infection in the CNS.

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W23 Methamphetamine Enhances HIV Infection of Human Monocytes

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Methamphetamine (METH), a potent addictive psychostimulant, is highly prevalent in HIV-infected individuals. METH has been demonstrated to compromise the host innate immunity and enhance HIV infection of various cell types, including monocytes-derived dendritic cells, macrophages, CD4+ T cells and neural progenitor cells. In the present study, we examined the impact of METH on HIV infection of primary human monocytes. We found that comparing to untreated monocytes, METH-treated cells showed 2-3-fold increase in HIV p24 protein and GAG gene expression, respectively. Mechanistically, METH treatment of monocytes resulted in the inhibition of the antiviral interferon (IFN)-stimulated genes (ISGs: OAS2, GBP5, ISG56, Viperin and ISG15) and the HIV restriction miRNAs. In addition, METH could suppress the expression of several key regulators in IFN signaling pathway, including RIG-I, IRF7 and STAT1. These observations indicate that METH impairs the intracellular innate immunity in monocytes, providing a favorable microenvironment for HIV replication and persistence.

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W24 In vitro validation of CRISPR-Cas9 mediated targeting of transgene integration sites to excise HIV-1 sequences from HIV-1 Tg rat

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Persistent expression of HIV-1 proteins in the absence of active viral replication in HIV-1 Tg rat model resembles pathologies observed in ART-controlled HIV-1 positive patients. HIV-1 transgene expression in different tissues leads to progressive HIV-1 proteinotoxicity, resulting in metabolic and behavioral malfunctions, which can be further intensified by the use of addictive substances. Recently whole genome sequence analysis was performed leading to identification of the sites of HIV-1 transgene integration in the HIV-1 Tg rat genome. Using this information, we developed a PCR based genotyping and qPCR techniques to accurately identify and quantify transgene positive cells/tissues. The two HIV-1 transgene integration sites in chromosome 10 and 13 were confirmed by Sanger sequencing of PCR amplicons spanning rat and HIV-1 sequences. Based on these data we were able to develop a new, simplified CRISPR-Cas9 mediated transgene excision strategy. Two pairs of gRNAs each targeting 5' and 3' flanking regions of integration sites in chromosome 10 and 13 were designed and cloned into AAV and lentiviral delivery vectors. Next, the constructs were tested in vitro using primary rat embryo fibroblasts derived from the single litter of HIV-1 Tg rats and rat neuronal cell line. CRISPR-Cas9 mediated excision of transgene sequences resulted in inhibition of HIV-1 expression in treated cells. Successful removal of transgene sequences from different tissues and organs of transgenic animals should limit and/or reverse HIV-1 protein expression mediated pathologies.

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W25* Effect of HIV-1 Tat-induced senescence on astrocytes and BMECs in a blood-brain barrier model

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Although anti-retroviral therapy (ART) has decreased the severity of neurocognitive impairment in human immunodeficiency virus type 1 (HIV-1)-infected patients, more than 50% of these individuals still suffer from some form of impairment. The driving force behind this observation is still unclear. Notably, the severity of impairment has been shown to be correlated to the level of chemokines, inflammation, and activated cells, rather than with the number of HIV-1-infected cells in the brain. Factors involved in the onset of neurodegenerative diseases have been linked to neuro-inflammation and altered blood-brain barrier (BBB) integrity and may be mediated by HIV-1 viral accessory proteins. The HIV-1 protein Tat has been shown to cause neuropathologic changes, inflammation, increased BBB permeability, and inhibition of apoptosis in the CNS, all of which are also features of cell senescence. Senescence is a cell mechanism usually involved in aging that is driven by telomere shortening and oxidative stress and results in a permanent exit of the cell cycle with resistance to apoptosis. We have shown that brain microvascular endothelial cells (BMECs) and astrocytes treated with Tat had increased SA-beta gal-positive staining, a biomarker of senescence, indicating that Tat exposure to cells of the BBB can result in senescence. When senescence occurs prematurely, it has been shown to be associated with neurodegenerative disorders, and it is likely that Tat-induced premature senescence of cells of the BBB could also be linked to neurocognitive impairment.

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W26* Role of dopamine-mediated conformational changes of CCR5 in HIV infection of macrophages

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Although combination antiretroviral therapy (cART) has greatly reduced HIV-related mortality, there are still 37 million people worldwide infected with HIV. Even with cART, infected cells within the central nervous system (CNS) can produce new virus and release neurotoxic factors that may contribute to NeuroHIV. Drug abuse, which increases CNS dopamine, is disproportionately prevalent within the HIV-infected population, and our lab has shown that dopamine increases HIV entry in macrophages, the primary target cell for HIV infection in the CNS. This suggests that increased dopamine from substance abuse could accelerate the spread of infection, but the mechanism is unclear. One explanation is dopamine-mediated changes in CCR5, the primary co-receptor used by HIV to enter macrophages. CCR5 can adopt several conformations to differentially regulate the accessibility or binding affinity to HIV, and different CCR5 subpopulations colocalize with lipid rafts, which can alter efficiency of HIV entry. Our data show that dopamine increases the relative abundance of different CCR5 conformations on the macrophage surface, and that colocalization of CCR5 subpopulations to lipid rafts may be altered by dopamine. Further, we show that these dopamine-mediated conformational changes can be altered by macrophage phenotype. Moving forward, we are focusing on determining dopamine-induced CCR5 epigenetic changes, and whether these changes in CCR5 play a role in dopamine-induced HIV entry. Understanding dopamine-driven CCR5 expression will reveal novel targets for HIV inhibition in drug abusers.

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W27* S-EQUOL RESTORES THE DEVELOPMENTAL TRAJECTORY OF TEMPORAL PROCESSING IN THE HIV-1 TRANSGENIC RAT

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Due to the sustained prevalence of HAND in the post-cART era, and its characterization as a neurodegenerative disease, there is a critical need to develop adjunctive therapeutics targeted to alter the trajectory of neurocognitive impairments (NCI). To address this knowledge gap, the present study examined the utility of S-equol, a phytoestrogen produced by gut microbiota, as an innovative therapeutic strategy. HIV-1 transgenic (Tg) and F344/N control animals were treated with 0.2 mg S-equol or placebo daily following neurocognitive testing during a formative period (i.e., Postnatal Day (PD) 28 to PD90). Temporal processing, a potential elemental dimension of HAND, was assessed using two experimental paradigms every 30 days from PD60 to PD210. First, in visual prepulse inhibition, a shift in the point of maximal inhibition supports faster temporal processing development in HIV-1 Tg animals treated with S-equol (PD120) relative to HIV-1 Tg animals treated with placebo (PD150). Second, in gap-PPI, a difference in the best-fit function (i.e., HIV-1 Tg S-equol: Quadratic, R2=0.97; HIV-1 Tg Placebo: Segmental Linear Regression, R2=0.93) provides further evidence for enhanced development of temporal processing in HIV-1 Tg animals treated with S-equol; an enhancement which resembles the developmental trajectory of temporal processing in control animals. Thus, the present study supports the utility of S-equol as an efficacious therapeutic for altering the trajectory of NCI in the HIV-1 Tg rat, and more broadly, of targeting gut microbiota as a mechanism to modulate NCI in HIV-1.

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W28 Examining unique naturally occurring HIV-1 Tat truncations obtained from patient peripheral blood samples in the Drexel Medicine CARES cohort

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Human immunodeficiency virus type 1 (HIV-1) encodes for Tat, a multifunctional regulatory protein that is involved in transcriptional enhancement and in causing neurotoxicity and central nervous system (CNS) dysfunction. This study examines HIV-1 subtype B Tat sequences from 2006 to 2015 within the Drexel University College of Medicine CNS AIDS Research and Eradication Study (CARES) cohort to understand Tat length in patients. The Los Alamos National Laboratory (LANL) database was used as a comparator. Miscoded stop codons were present in CARES and LANL sequence databases and protein variability was highly similar. The majority of Tat sequences in CARES and LANL are 101 residues in length. Unique Tat lengths in CARES and LANL databases were 31, 36, and 39 residues in length. When CARES patients were longitudinally examined, sequence lengths of 101 had a low probability of reducing to below 48, and sequences had a high probability of increasing to above 86 during their next visit, when below 48 residues in length. There was no observed correlation between Tat lengths observed and clinical parameters within the LANL or CARES cohorts. Preliminary results have suggested that the truncated Tat constructs were able to transactivate the LTR, even though they do not have a complete transactivation domain, nor TAR binding domain. Current studies are being performed to determine whether the transactivation was due to direct or indirect mechanisms.

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W29 Ferritin accumulation in brains of HIV-1 associated neurocognitive disorder

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Iron plays in a wide array of cellular functions and is essential for normal brain physiology. However, iron overload in brain leads to cognitive impairment and contributes to the development of a variety of neurodegenerative diseases. Recent studies have shown that altered iron homeostasis is involved in HIV-1 disease progression and the development of HIV-1 associated neurocognitive disorder (HAND). We determined the levels and distribution of ferritin, the iron storage protein, in postmortem brain tissues from HIV-1 patients with normal cognition (HIV-CN) or with HAND. We demonstrated that the levels of ferritin heavy chain (H-ferritin) were markedly increased in HAND brain. Increased H-ferritin signal is observed in MAP2-positive neurons, which correlates with degeneration feature of these neurons. In addition, highly dense H-ferritin signals were observed in a set of cells that is MAP2-negative. We observed that highly dense H-ferritin signals co-localized with GAD67-positive interneuron, but not with parvalbumin-positive interneurons, GFAP-positive astrocyte, or IBA1-positive microglia. Given that lysosomes are involved in iron release from ferritin, we also assessed the

structure of lamp1-positive lysosomes. We demonstrated that lamp1-positive lysosomes are enlarged in HAND brain but not HIV-CN. Significantly, H-ferritin signals co-localized with lamp1-positive signal in both MAP2-positive neurons and in GAD67-positive interneuron. Our findings suggest lysosome dysfunction contribute to the accumulation of ferritin in neurons and development of cognitive impairment.

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W30 Dopamine modulates HIV entry into macrophages via Gq-dependent signaling mechanisms

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Despite the use of combined anti-retroviral therapy, many individuals with HIV still experience neurocognitive decline and neuropathology, particularly in dopaminergic regions of the CNS. This suggests that dopamine may play a role in the development of NeuroHIV. Our lab has shown that dopamine can increase HIV entry and replication in primary human macrophages, the primary cell type infected in the CNS. However, mechanisms by which dopamine mediates these effects remains unclear. We demonstrated that activation of both D1-like and D2-like receptors increases viral entry into macrophages, suggesting these receptors may act through a common mechanism. Our current data show that dopamine stimulates calcium release in human macrophages, and inhibition of this calcium flux prevents dopamine-mediated increases in viral entry. This suggests that dopamine's effects on viral entry is modulated by signaling through its alternative pathway, wherein D1-like receptors couple to Gq and D2-like receptors couple to G-beta-gamma, leading to PLC activation and IP3-mediated calcium release. Additional data showing that dopamine receptor activation did not increase cAMP production, but stimulated calcium release and PKC phosphorylation, support this hypothesis. Further, the dopamine-mediated increases in both calcium release and PKC phosphorylation were partially inhibited by treatment with the Gq inhibitor YM-254893. These data suggest that in human macrophages, dopamine mediates its effects on viral entry, at least partially, via Gq-dependent signaling mechanisms.

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W31 Role of dopamine in the modulation of macrophage-mediated inflammation: implications for NeuroHIV and drug abuse

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Drug abuse is an important comorbidity in HIV infection and has been linked to changes in the development of HIV-associated Neurocognitive Disorders (HAND). All drugs of abuse increase extracellular dopamine in the CNS, and research suggests that inflammation induced by elevated dopamine could enhance the development of HIV-associated neuropathology. However, the precise mechanism(s) by which elevated dopamine could exacerbate the progression of HAND remain unclear. As the primary targets for HIV in the CNS are myeloid cells, the effects of dopamine on these cells may be a key connection between dopaminergic changes and HIV-associated neuroinflammation. Our data show that dopamine treatment of human macrophages promotes inflammation by inducing production of the inflammatory mediators IL-1b, IL-6, IL-18, CCL2, CXCL8, CXCL9, and CXCL10. Further, dopamine-mediated modulation of specific cytokines is correlated with macrophage expression of dopamine-receptor transcripts, particularly DRD5, which we show to be expressed at significantly higher levels than other dopamine-receptor subtypes. These effects may be induced by activation of inflammatory pathways, as our data show dopamine activates the NF-kB pathway. This results in increased expression of NF-kB modulated genes including NLRP3 and IL-1b that prime the NLRP3 inflammasome complex. Thus, elevated CNS dopamine may potentiate neuroinflammation via the NF-kB pathway. Overall, these data will provide more understanding of the role of dopamine in the development of NeuroHIV, and may reveal new therapeutic targets for HAND.

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W32* The blood-brain barrier is dysregulated in the hippocampus of meth self-administering HIV-1 transgenic rats

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The neurological profile of HIV/AIDS has improved with cART, which controls plasma viral load and prolongs life. Yet, the brain remains a viral reservoir and neurological disorders, including hippocampal-mediated memory impairments, are associated with the increased life span. The blood-brain barrier (BBB) is the primary route by which HIV gains access to the brain. Abuse of methamphetamine (meth) can damage the BBB, but effects of HIV-meth co-morbidity on the BBB and the hippocampus are unclear. To address this knowledge gap, we trained HIV-1 transgenic (Tg) and non-Tg rats to self-administer meth (0.02-0.04mg/kg/0.05ml iv infusion, 2h/day for 21 days). Cumulative meth intake was 4.5 Â±0.3 and 5.2Â±0.5mg/kg, respectively. We evaluated BBB tight junction proteins occludin and claudin-5 in the hippocampus. To indicate a mechanism that may contribute to the BBB dysregulation, we assessed MMP9 levels. Two way ANOVA revealed a genotype effect for occludin and claudin-5, but no meth effect. Post hoc analysis revealed differences between saline Tg and saline non-Tg for both proteins, and meth non-Tg and saline non-Tg rats for occludin. There was an overall genotype effect for MMP9, but no meth effect. Post hoc analysis revealed differences between saline Tg and saline non-Tg, and meth non-Tg and saline non-Tg rats. Evaluations of protein markers of two signaling pathways that regulate MMP9 transcription; NFÎ°B and MAPK (ERK), revealed an overall genotype effect for NFÎ°B but not the MAPK pathway. Hippocampal BBB dysregulation by HIV-1 proteins and meth may involve NFÎ°B/MMP9.

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W33 Lipocalin-2 deficiency protects against neuronal damage and behavioral deficits in a CCR5 dependent manner in a transgenic model of HIV-induced brain injury

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Infection with human immunodeficiency virus (HIV)-1 remains a serious threat to global health. Up to 50% of HIV patients develop some form of neurological and neurocognitive complication categorized as HIV-associated neurocognitive disorders (HAND). The pathological mechanisms leading to HAND, however, remain incompletely understood. We have recently shown in vitro that the acute phase protein lipocalin-2 (LCN2) exerts two seemingly paradox functions: i) it is neurotoxic in a CCR5-dependent fashion, and ii) it is necessary for maraviroc, an

inhibitor of the chemokine receptor CCR5, to indirectly protect against neuronal damage by a CXCR4-preferring HIVgp120. To explore the role of LCN2 alone and in combination with CCR5, we cross-bred transgenic (tg) mice expressing HIV envelope protein gp120 in the central nervous system (HIVgp120tg), which display features observed in NeuroHIV patients, with a genetic knockout of LCN2 (LCN2ko) or a double knock out for CCR5 and LCN2 (DKO). Histopathological analysis and behavioral test show that animals lacking only LCN2 are largely protected against damage by HIVgp120; however, animals lacking both CCR5 and LCN2 show behavioral deficits and neuronal damage. Altogether, our findings provide evidence for a functional interdependence between LCN2 and CCR5 since deficiency of each alone, but not in combination, results in protection against HIVgp120. A better understanding of this interplay mechanism may thus help the development of new treatments for NeuroHIV.

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W34 Toll-like receptor 3 regulates Zika virus infection and associated host inflammatory response in primary human astrocytes

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The connection between Zika virus (ZIKV) and neurodevelopmental defects is widely recognized, although the mechanisms underlying the infectivity and pathology in primary human glial cells are poorly understood. Here we showed that three isolated strains of ZIKV, an African strain MR766 (Uganda) and two closely related Asian strains R103451 (Honduras) and PRVABC59 (Puerto Rico) productively infect primary human astrocytes, although Asian strains showed a higher infectivity rate and increased cell death. Inhibition of AXL receptor but not the Tyro3 receptor significantly attenuated viral entry, suggesting an important role of AXL receptors in ZIKV cell entry, irrespective of lineage. Infection by PRVABC59 elicited the highest release of inflammatory molecules including RANTES, IP-10, and IFN-β. Activation of the autophagy pathway was evident with increased expression of the autophagy-related proteins Beclin1, LC3B and p62 with all three strains of ZIKV. Pharmacological inhibition of the autophagy pathway and genetic inhibition of Beclin1 showed minimal effects on ZIKV replication, however, Beclin1 silencing caused increased secretion of inflammatory molecules. On the other hand, pharmacological and genetic inhibition of toll-like receptor 3 (TLR3) caused a decrease in viral titers and in the viral-induced inflammatory response in infected astrocytes. We conclude that TLR3 plays a vital role in both ZIKV replication and viral-induced inflammatory responses, irrespective of the strains, while the autophagy protein Beclin1 influences host inflammatory responses.

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W35* Retinoic Acid (RA) enhances the Recovery of Replication Competent Virus from Latent SIV infected cells by activating α4β7-expressing cells

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Objective: The accurate estimation and eradication of HIV viral reservoirs is limited by incomplete reactivation of cells harboring latent replication competent virus. We investigated whether addition of retinoic acid (RA) improves: (a) the quantification of latent virus following stimulation with a T-Cell activator (b) SIV replication in-vivo. Design and methods: Naïve and ART treated SIV infected rhesus macaques were a) treated in-vivo with RA (b) PBMCs collected and activated in-vitro using anti-CD3/CD28 + IL-2 in the presence/absence of RA. Viral loads and p27 levels were quantified using RT PCR and ELISA. Flow cytometry was utilized to determine α4β7 expression levels and viral reservoirs estimated using RA - modified QVOA and TILDA assays. Results: The addition of RA enhanced >3-fold increase in the density of α4β7 hi expression on CD4+ T cells. There was also a 3- and 5-fold increase, of reactivation of the replication competent viral reservoir and p27 levels respectively in RA-treated activated cells compared to cultures without RA. Finally, the in-vivo administration of RA to macaques led to moderate increases in plasma viral loads. Conclusions: Targeting the RA pathway can be a useful approach to improve the efficiency of current methods used to upregulate α4β7 expression and activate latent reservoirs.

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W36 Neuroimmune Consequences of Heroin Withdrawal on Stress Enhanced Fear Learning

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Multiple studies suggest heroin use is associated with increased anxiety and severity of PTSD symptoms, while opioid withdrawal produces similar symptoms to those of PTSD. This suggests a common underlying neurobiological mechanism for both opioid withdrawal and PTSD. Our laboratory has examined the neural mechanisms of stress enhanced fear learning (SEFL), an animal model of PTSD. We have established the severe stressor in SEFL induces a time-dependent, region and astroglial-specific increase in dorsal hippocampus (DH) interleukin-1B (IL-1B). To gain insight into the relationship between PTSD and heroin withdrawal, our laboratory conducted two sets of experiments. The first used a chronic, escalating heroin dose regimen and withdrawal paradigm in place of the severe stressor in SEFL to examine the effects of heroin withdrawal on fear learning. We found escalating heroin administration and withdrawal substitutes for the severe stressor as both severely stressed and heroin withdrawn animals exhibited enhanced fear leaning following these experiences. The second set of experiments explored whether heroin withdrawal produced DH neuroimmune alterations. Protein analysis of IL-1B and glial fibrillary acidic protein (GFAP), a marker of astrocyte reactivity, following heroin withdrawal revealed increases in both DH IL-1B and GFAP expression. These studies provide evidence that chronic, escalating heroin administration and withdrawal is comparable to the severe stressor in SEFL, produces a PTSD-like phenotype in an animal model, and elevates DH IL-1B and GFAP protein levels.

W37* Poly-lactic-glycolic-acid (PLGA) mediated gene delivery to astrocytes requires arginine modified poly-ethyl-imine (PEI) polymer to facilitate gene expression

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Astrocyte dysfunction is a hallmark of central nervous system injury or infection and is rapidly being considered as a primary contributor to neurodegeneration. Chronic inflammation during HIV-1 infection, impairs

astrocyte release of a key neuroprotective factor, tissue inhibitor of metalloproteinases (TIMP-1), exacerbating HIV-1 associated neurocognitive disorders (HAND). Gene therapy has arisen as an innovative technique that provides excellent prospect for disease intervention. Poly(lactic-co-glycolic-acid) (PLGA) and poly-ethyl-imine (PEI) are both common constructs used in gene delivery manifesting their own set of pros and cons. PLGA nanoparticles are FDA approved and well established for their biocompatibility while PEI-polymers illustrate high delivery efficiency but can cause cytotoxicity. The current study investigates PLGA-mediated gene delivery to primary human astrocytes. Yoyo-labeled luciferase DNA encapsulated in PLGA is released in the cytoplasm but did not localize to the nucleus. Co-treatment with arginine modified PEI polymers (AnPn), resolved the failure of PLGA-mediated gene delivery to the nucleus by enhancing the stability of cytoplasmic DNA resulting in efficient luciferase gene expression. Co-treatment biocompatibility was confirmed in both astrocytes and neurons via LDH and MTT activity. Our discovery identifies an improved model providing an efficient, biocompatible, and clinically translatable treatment for astrocyte-targeted gene therapy to restore astrocyte function during HAND.

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W38 Metformin Enhanced HIV Gene Expression and Production

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HIV-1 induces mTORC1 activity and hijacks the Rag GTPase/mTORC1 complex for optimal expression of the structural protein Gag and virus trafficking. Meanwhile, Metformin has been shown to activate AMPK and inhibit mTORC1. Metformin's antiviral effects against hepatitis B, dengue, and Zika viruses have been well documented. These findings led us to hypothesize that metformin inhibits HIV replication, release, and trafficking through inhibition of mTORC1 activity. We used HEK293 and TZM-bl cells to determine the effect of metformin on HIV gene expression and virus production. MTT assay was used to determine cell viability, and reverse transcriptase (RT) assay was used to determine virus replication and production in HIV transfected cells treated with various concentrations of metformin. Western blotting was performed to determine intracellular HIV protein expression. No effects on cell proliferation were noted in both 293T and TZM-bl treated with up to 4 mM Metformin. Metformin did not alter HIV LTR promoter activity although it led to increased virus production. Consistent with this finding, Metformin increased intracellular expression of Gag and Tat. Metformin did not appear to have any effects on the activity of HIV reverse transcriptase. These findings demonstrate that Metformin enhances HIV gene expression and production and suggest that Metformin may regulate steps of the HIV life cycle other than RT and HIV LTR promoter transcription. These findings also provide evidence to call for caution about the use of Metformin in treating HIV-infected diabetic individuals.

W39 Beta adrenergic receptor blockage inhibit inflammation caused by the HIV-1 protein Nef

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Nef is an early HIV-1 protein produced by latently infected astrocytes. Our model in Sprague Dawley (SD) rats shows Nef causes memory impairment, inflammatory cell infiltration, blood brain barrier (BBB) compromise and peripheral organ inflammation. In this study we examine the role of astrocyte Nef expression and the activation of the sympathetic nervous system (SNS). We infused male and female SD rats with astrocytes transfected to produce Nef and divided into four groups: Nef, Nef with propranolol, propranolol-only and naive. The beta-adrenergic antagonist propranolol (10mg/kg) was administered for three days starting one day prior to surgery. Estrus cycle in female rats was tracked using smears. Two days after surgery, the rats were sacrificed, blood and spleen were collected to performe ELSIA of IL-1α and flow cytometry for CD68 and iNOS on splenocytes. We found no effect of treatments on estrus cycle. Levels of IL-1α were higher in male rats and female rats and treatment with propranolol restored to basal levels. In females interestingly, we found a subdivision in the Nef-treated rats expressing very high levels of IL-1α. Analysis of the splenic leukocytes by flow cytometry demonstrated that CD68 and iNOS were upregulated by Nef; this effect was also restored to baseline levels in the Nef + propranolol groups (males and females). These results indicate involvement of the adrenergic system in mediating the immune response that Nef caused after expression in the brain astrocytes.

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W40 Drug Resistance Mutations Insight on HIV-1 Patients from Haiti using Genotypic and Phylogeny Analysis

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The Human Immunodeficiency Virus (HIV) ranks among the top ten leading causes of death in low-income countries around the world. More than 150,000 patients are currently diagnosed with HIV infections in Haiti. Haiti's economic status has become a limitation for patients to get treated and acquire drug resistance mutations analysis. We aimed to provide an updated insight on how HIV-1 has mutated over the past years studying samples from patients in Haiti. We hypothesized that due to the lack of strict treatment regiments, and the fact that they are only using Retro-Transcriptase (RT) inhibitors as a first-line regimen; the RT region of the pol genome would be highly mutated, and the Protease region would not be mutated. To test our hypothesis, we extracted the viral RNA, which allowed us to obtain the RT and Protease regions of HIV-1. Then, we amplified the samples and moved on to genotyping protocols. Results showed that samples contained either a non-nucleoside retro-transcriptase inhibitor (NNRTI's) resistance mutation or nucleoside retro-transcriptase inhibitor (NRTI's) resistance mutation. Also, that one sample could count with various resistance mutations on the RT region, which means that the samples were highly mutated in that specific region. These results suggest that treatments to inhibit the Retro-Transcriptase activity in HIV-1 patients from Haiti are failing. However, none of the studied samples showed protease region mutations, which means that the samples are susceptible to any treatment that contains protease inhibitors.

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W41 CB2R agonist, JWH-133, decreases HIV/MDM-induced CATB release and neuronal death

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HIV-associated neurocognitive disorders (HAND) are prevalent despite combined antiretroviral therapy (cART), affecting 53% of people living with HIV worldwide. Our laboratory has demonstrated increased expression of cathepsin B (CATB), a lysosomal enzyme, in postmortem brain tissue with HIV encephalitis (HIVE) and HAND. Also, we have demonstrated increased secretion and neurotoxicity of CATB, from in vitro HIV-infected monocyte-derived macrophages (MDM). In a search for possible therapies, activation of cannabinoid receptor type 2 (CB2R) by agonists

has been shown to inhibit HIV-1 replication in MDM, microglia and CD4+T cells. Also, it reduces pro-inflammatory cytokines release and the neurotoxicity caused by HIV-1 viral proteins. However, the effect of CB2R activation on CATB secretion and neurotoxicity from HIV-infected MDM is unknown. We hypothesize that CB2R agonist, JWH-133, will decrease HIV/MDM-induced CATB release and neuronal apoptosis. MDM were isolated from healthy donors, inoculated with HIV-1ADA, and treated with the CB2R agonist, JWH-133. HIV-1 p24 and total CATB levels were determined from supernatants of HIV-infected MDM at the end of cultures using ELISA. Cell viability was determined by MTT assay. Neuronal apoptosis was assessed using the TUNEL assay. Results show that JWH-133 reduces HIV-1 replication and CATB secretion in a dose-dependent manner. Also, JWH-133 decreased HIV/MDM-induced neuronal apoptosis. Our results suggest that agonists of CB2R represent a potential therapeutic strategy against HIV/MDM-induced CATB neurotoxicity and HAND.

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W42 Mechanisms of white matter loss due to antiretroviral, elvitegravir

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The thinning of the corpus callosum and disruption of white matter microstructures in combined antiretroviral (ARV) therapy (cART)-treated HIV+ patients suggest that ARVs perturb myelin production and oligodendrocyte (OL) maturation. We hypothesized that exposure of OLs to ARVs alters their maturation, influencing HAND persistence in the cART era. We stimulated primary rat OL precursor cells (OPCs) to differentiate into mature OLs with concurrent exposure to ARVs: elvitegravir (EVG), raltegravir (RAL) or cobicistat (COBI), the bioavailability boost. OPC maturation was inhibited by EVG, but not by RAL and COBI. Pretreatment with an integrated stress response inhibitor, trans-ISRIB, prior to EVG, rescued OL maturation. To examine the effect of ARVs on remyelination, we treated mice with cuprizone, a demyelinating compound, for five weeks and allowed them to recover for three weeks or treated them with daily intrajugular injection of EVG and COBI during the three-week recovery phase. Sections of corpus collosum were stained for myelin by luxol fast blue (LFB), mature OLs by ASPA, and GFAP and IBA1 to assess neuroinflammation. EVG-treated cuprizone recovery mice displayed failure of remyelination indicated by reduced ASPA and LFB staining. Persistent neuroinflammation was evident in the corpus callosum in these mice as compared with the vehicle-treated controls. These data suggest that EVG inhibits OL maturation in vitro and in vivo and illustrate the need for further investigation of ARVs on myelin changes and neurocognitive impairment persistent in HIV+ patients.

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W43* Role of extracellular vesicles in HIV-1 and methamphetamine induced neurotoxicity

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The advent of combined antiretroviral treatments (cART) has markedly decreased the prevalence of HIV-associated dementia. However there remains a high prevalence rate of the milder forms of HIV-associated neurocognitive disorders (HAND). Although many contributing factors have been studied, the role of drugs of abuse has remained elusive. Methamphetamine (meth) and related amphetamine compounds, which are potent psychostimulants, are among the most commonly used illicit drugs. Long-term meth abuse is associated with a host of systemic and neurological maladies. However, the mechanisms underlying meth and HIV neurotoxicity are still not known. This study focuses extracellular vesicles (EVs) and their role in HIV infection and chronic meth abuse. Our results presented here, indicate that meth not only increases EV biogenesis and release but can also change the composition of EV cargo. Nanoparticle tracking analysis and transmission electron microscopy revealed that meth changed EV dynamics in uninfected and HIV infected macrophage cultures. Our investigation revealed that the genes involved in the endosomal sorting complexes required for transport (ESCRT) are responsible are significantly increased upon meth treatment. Further, our data reveals that meth increases the release of HIV accessory protein, myristoylated Nef (Myr-Nef), that plays a critical role in HIV/AIDS progression. Furthermore, we also reveal that gp120 is released in the EVs along with Myr-Nef. We conclude that chronic meth abuse interferes with EV biogenesis and cargo release in HIV infected cells.

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W44* Effect of IFNAR1 Deficiency on Neuronal and Astrocytic Cell Marker

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Infection with HIV-1 often causes brain injury, which presumably underlies HIV-associated neurocognitive disorders (HAND). The pathogenesis of HAND is not completely understood. Our laboratory uses a transgenic mouse model of HIV-associated brain injury expressing the viral gp120 envelope protein in the central nervous system. These HIVgp120tg mice display key neuropathological features of human HIV brains, including loss of microtubule-associated protein (MAP)-2 positive neuronal dendrites and elevated expression of astrocytic glial fibrillary acidic protein (GFAP) when compared to non-tg controls. The innate immune system responds to HIV-1 infection with the production of interferons (IFNs). Type I IFNs (IFNα and IFNβ) exert their biological functions through interaction with the type I IFNs receptors (IFNAR1 and -2). We recently showed that IFNβ prevented in vitro and in vivo neuronal injury induced by HIVgp120. Neuroprotection by IFNβ was dependent on IFNAR1. Our present study investigated the role of IFNAR1 in HIV induced brain injury by cross-breeding IFNAR1 knockout (IFNAR1KO) with HIVgp120tg mice. The brains of one-year-old IFNAR1 deficient and wild-type gp120tg mice and non-tg controls were analyzed using quantitative fluorescence microscopy. Our data shows that knocking down IFNAR1 results in reduced neuronal MAP-2 in the cerebral cortex and hippocampus with no further drop off due to HIVgp120. In addition, IFNAR1 deficiency does not show any protection against gp120-induced astrocytosis and appears to diminish the astrocytic GFAP expression in the hippocampus.

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W45 Expression of HIV-1 Tat in transgenic mice decreases [3H]dopamine uptake via both dopamine and norepinephrine transporters in the prefrontal cortex

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Abnormal dopaminergic transmission has been implicated as a mediating factor of HIV-1 associated neurocognitive disorders. Our laboratory has demonstrated that in vitro HIV-1 Tat protein reduces dopamine transporter (DAT) function via a direct allosteric interaction. This study determined whether the inhibitory effects of Tat on DAT function could be replicated in an inducible Tat transgenic (iTat-tg) mouse model. Additionally, both DAT and the norepinephrine (NE) transporter (NET) are responsible for the reuptake of dopamine in the prefrontal cortex (PFC), which is associated with cognitive brain function. Following 7-day administration of doxycycline (Dox) or saline, the maximal velocity (Vmax) of [3H]dopamine uptake via DAT and NET in PFC of Dox-treated iTat-tg mice was decreased by 27% and 34%, respectively, compared to their saline controls, whereas after 14-day Dox treatment, the Vmax of [3H]DA uptake via DAT and NET in the PFC by 34% and 22%, respectively. No differences in the Vmax were found between Dox and saline treated control C57BL/6J mice. The decreased Vmax of [3H]DA uptake was not accompanied by changes in total DAT or NET expression. Whole-cell electrophysiology showed an reduction of action potential output of nucleus accumbens shell medium spiny neurons as well as PFC pyramidal neurons of Dox-treated iTat-tg mice relative to saline controls. These findings add to the evidence that in vitro Tat-induced inhibition of DA transport can be documented in iTat-tg mice, which may be responsible for the neurocognitive deficits observed in HAND.

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W46 Plasma Biomarkers as Indicators for Neurocognitive Impairment in HIV+ Individuals

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Chronic inflammation in HIV patients correlates with the severity of HIV-associated neurocognitive disorders (HAND), suggesting that inflammatory mediators could be indicators for the progression of HIV-associated neurocognitive impairment. The current clinical evaluations for HAND are designed for diagnosis after the onset of the disorder, limiting avenues for intervention. Therefore, there is a need for prognostic biomarkers to determine the likelihood for HAND development. We hypothesize that inflammatory factors from blood plasma and peripheral blood mononuclear cell (PBMC) can serve as prognostic markers of HIV-associated neurocognitive impairment and these associations may be dependent upon race and sex. Performance in seven neurocognitive domains was measured in 121 HIV+ male and female African, Caucasian and Hispanic Americans using CNS Vital Signs computerized tests. Inflammatory factors were measured in participant plasma and PBMCs and association to neurocognitive tests were identified by a multivariate multiple linear regression model. Here, we demonstrate that higher plasma levels of monocyte chemoattractant protein 2 and tissue inhibitor of metalloproteinases 1 significantly associate with lower neurocognitive scores in all domains tested except reaction time. In addition, higher plasma levels of chemokine c-c ligand 17, interleukin (IL) 10, and IL-23 significantly associate with lower neurocognitive scores in processing speed and executive functioning. Further studies to characterize these markers in PBMCs and as predictors of HAND are being conducted.

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W47 HIV Infects Platelets in Treatment Naïve Viremic Patients

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Platelets are small annucleate cells primarily known for their role in maintaining hemostasis. However, there is emerging evidence that they also function in an immune capacity, including complex formation with monocytes and CD4+ T cells. Previous in vitro studies implicate that platelets interact with and engulf HIV virions. We further investigated platelet-virus interactions to ascertain whether or not they occur in persons with HIV. We confirmed previous findings of in vitro interaction through co-culturing platelets from healthy donors with HIV. We identified virus-like particles and viral RNA inside of platelets using electron microscopy and PCR respectively. Furthermore, we confirmed this interaction using immunostaining for glycoprotein gp120. To validate these findings in vivo we isolated platelets from cART-naïve viremic patients and performed confocal microscopy and imagestream flow cytometry. We found evidence of virions associated with and inside of platelets from these infected individuals.This association is significantly decreased after patients have been on antiretroviral therapy for 3 months.\

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W48 Molecular Dynamics of amyloid-β protein binding with the potential neuroprotectant, Withaferin A

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The abnormal processing of Amyloid precursor protein (APP) by β-secretases (beta) and $\hat{1}^3$-secretases (gamma) leads to anomalous production and aggregation of Aβ. This aggregation is neurotoxic and leads to neurological complications. Our previous study demonstrated that, a novel plant based drug compound WA (Withaferin A), is a potential neuroprotective agent which neutralizes the toxicity induced by Aβ secretion in-vitro. Moreover it is imperative to understand the mechanism of action of WA, and focus on its specificity to the Aβ secreted by the neurons in the diseased scenario. Present work focuses on the molecular mechanism of interaction of WA with Aβ. It comprises of a Bio-computational study carried out with the aim of exploring the binding/acting capability of WA, a steroidal lactone, as a ligand to Aβ using molecular docking and molecular dynamics simulation studies. We modelled the Aβ monomer protein with the aid of Molecular Dynamics Software and further docked WA drug molecule employing the Rosetta software. We were able to demonstrate the binding affinity of WA to Aβ. The designed protein model was visualized by Chimera X protein structure visualization software, which showed that the drug molecule binds to the Aβ protein in the middle region of the protein. The amino acid motif involved with the binding to the WA was FAEDVGS highlighting the mid-region amyloid-β capture by WA. Three stable Hydrogen bonds were formed between WA and the amino acids, ASN17, GLY15 and SER16 of Aβ protein. Aβ capture by neuroprotective agent and its stability is explained in this work

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W49* HIV alters Occludin-Alix-Cav-1 interactions in human brain vascular pericytes

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HIV enters the brain early after infection, causing disruption of the blood-brain barrier (BBB) by functional and structural hindrance of tight junctions (TJs). Occludin and caveolin-1 (cav-1) have been described as critical determinants of BBB permeability by regulating TJ protein expression. In addition, Alix is an early-acting ESCRT factor associated with membrane proteins that is involved in HIV budding from the cells. While the impact of HIV on BBB endothelial cells and astrocytes have been thoroughly studied, the role of pericytes during HIV infection remains largely undescribed. We propose to study the relationship between the integrity of this protein complex and HIV replication in pericytes. We demonstrated that occludin and Alix form stable complexes. We also discovered another interaction partner, cav-1. To understand the molecular interactions within this complex, we focused our study on the impact of individual components. Pericytes were transfected with an occludin or Alix expression vector, and siRNA cav-1. Next, cells were either infected or not with HIV for 48h. The expression of individual proteins was evaluated by Western Blot. We observed that occludin overexpression decreased cav-1, but not Alix, protein expression. We also described that cav-1 knockdown increased occludin, but not Alix, protein levels. Interestingly, we found that HIV attenuates occludin regulation by cav-1, and cav-1 knockdown does not increase occludin protein level when cells are infected with HIV.

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W50 Correlation of Serum Estradiol 17-β with HIV-infection and Cathepsin B Secretion in Macrophages

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High serum levels estradiol 17-β of E2 among HIV-1 seropositive women correlated with lower levels of viral infection, lower brain inflammation, and better cognitive performance. Furthermore, elevated levels of E2 impairs HIV-1 infection in macrophages (MDM) and minimize their cellular-related toxicity in vitro. We have demonstrated that a lysosomal cysteine protease, namely cathepsin B (CATB), is secreted from HIV-infected MDM and promotes neuronal apoptosis in vitro. Correlation of secreted CATB with MDM infection levels is unknown. We hypothesized that higher levels of E2 correlate with low levels of infection and decreased CATB secretion. For this matter, we quantified total E2 levels from serum plasma from 11 subjects, eight women and three seronegative men by ELISA. Similarly, we isolated monocytes from seronegative donors, infected them with HIV-1ADA till twelve days post-infection. Levels of HIV-p24 and CATB were quantified from MDM supernatants by ELISA. We demonstrated that infected MDM from women had reduced levels of CATB when compared to men (*p=0.020). Based on Pearson correlation, high serum levels of E2 correlated with lower p24 (p=0.07) and CATB levels (*p=0.020) in women. These E2 serum levels indicate that women were under their late follicular phase of the menstrual cycle (150-800 pg/mL). In men, very low E2 serum levels did not correlate with CATB (p=0.18) or p24 levels (0.57). These results indicate that E2 ameliorates HIV infection and CATB secretion. This information could to provide better therapies for HIV-1 infected women.

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W51 Exosomes Transport Anti-HIV Factors from Human Cervical Epithelial Cells to Macrophages

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The female reproductive tract (FRT) is a major site of HIV sexual transmission. As the outermost layer of cells in the FRT, the human cervical epithelial cells (HCEs) have direct contact with HIV or infected cells. Our early work showed that supernatant (SN) from TLR3-activated HCEs could efficiently inhibit HIV replication in macrophages. However, it remains to be determined how HCEs transport the anti-HIV factors to macrophages. The present study thus examined the role of exosomes in HCEs-mediated anti-HIV activity. We found that TLR3-stimulated HCEs could release the exosomes that contained a number of cellular antiviral factors, including several key IFN-stimulated genes (ISGs: ISG56, OAS1, MxA, and Mx2) and the HIV restriction microRNAs (miRNA-28, miRNA-29 family members, and miRNA-125b). The depletion of exosomes from TLR3-activated HCEs SN diminished HCEs-mediated anti-HIV activity in macrophages, indicating that HCEs-derived exosomes are responsible for transporting the antiviral molecules to macrophages. These findings suggest a novel antiviral mechanism by which HCEs participate in FRT innate immunity against HIV infection. Further studies are necessary in order to develop exosome-based delivery system for treatment of HIV disease.

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W52 Cytosolic DNA Sensors Activation Inhibits HIV Infection in Macrophages

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The cytosolic DNA-sensing pathway is critical in the activation of the IFN-dependent antiviral innate immune response. We here examined whether activating the DNA sensors can inhibit HIV infection of macrophages. We observed that the stimulation of monocyte-derived macrophages with poly(dG:dC) or poly(dA:dT), the synthetic ligands for the DNA sensors, induced the expression of both type I and III interferons (IFNs). In addition, the DNA sensors activation resulted in induction of the multiple intracellular HIV-restriction factors, including IFN-stimulating genes (ISGs: ISG15, ISG56, Viperin, OAS2, GBP5, MxA, MxB and Tetherin) and the anti-HIV microRNAs (miR-29C, miR-138, miR-146a, miR-155, miR-198, miR-223). In addition, the DNA sensor signaling of macrophages upregulated expression of the CC chemokines, the ligands for HIV entry coreceptor CCR5. More importantly, poly(dG:dC) or poly(dA:dT)-treated macrophages were more resistant to HIV infection and replication than untreated cells. These observations for the first time demonstrated the importance of cytosolic DNA-sensing signaling pathway in the macrophage innate immunity against HIV, suggesting that the DNA sensors are a potential target for immune activation-based anti-HIV therapy.

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3) Posters - Thursday

T01* Inhibition of the DEAD Box RNA Helicase 3 prevents HIV-1 Tat- and cocaine-induced neurotoxicity by targeting microglia activation

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HIV-1 Associated Neurocognitive Disorder (HAND) is a common and clinically detrimental complication of HIV infection. Viral proteins including Tat, released from infected cells, cause neuronal toxicity. Substance abuse in HIV-infected patients greatly influences the severity of neuronal damage. To uncover potential targets for anti- HAND therapy, we employed an AI-based literature mining system we developed called MOLIERE: Automatic Biomedical Hypothesis Generation to uncover previously unknown associations of the human genes with the HAND. Evaluation and prioritization of the highest scoring genes potentially associated with HAND revealed Dead Box RNA Helicase 3 (DDX3). Importantly, a selective small molecule inhibitor of DDX3 helicase activity, RK-33, has been developed and shown to selectively kill tumors that depend on DDX3 without any observed systemic toxicity in animal models, making it an ideal probe to test the role of DDX3 in HAND. We show that RK-33 prevents the combined neurotoxicity of HIV Tat protein and cocaine in rat and mouse cortical cultures. Transcriptome analysis by RNA-seq of the treated cultures shows that the majority of Tat-activated transcripts are microglia-specific genes and that RK-33 blocks their activation. These genes include major microglial markers and regulators of microglia activation, such as CSF1R and CSF3R. The treatment with RK-33 inhibits Tat and a cocaine-dependent rise of the number and size of the microglia cells in cortical cultures. The elevation of the production of IL-6, TNF-alpha and MCP-1/CCL2 proinflammatory cytokines is also inhibited by RK-33 treatment. These findings suggest that DDX3 contributes to microglial activation triggered by the combined insults of Tat and cocaine, and that pharmacological inhibition of DDX3 may have the potential to treat not only HAND but other neurodegenerative diseases with pathological activation of microglia.

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T02* Characterization of differential monocyte subtype responses in cocaine-induced neuroinflammation

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Monocytes have generally been viewed as a single population, however, their heterogeneous nature suggests that the subtypes may be differentially affected by cocaine. We have found that acute administration of cocaine in vivo alters the ratio of circulating monocyte subtypes, decreasing non-classical monocytes while increasing classical monocytes. This altered immune profile was accompanied by an increase in brain immune infiltration as determined by flow cytometry. Although the increase in infiltration was small as compared to other conditions of sterile inflammation such as neurotrauma, even small increases can potentially lead to transient neurocognitive deficits or amplification of other conditions such as HIV-associated neurocognitive disorders. Further investigations found that the administration of cocaine to isolated human monocytes showed a subtype differential response with increasing cell death only of non-classical monocytes that was reversed by blocking a known cocaine target, sigma1 receptor. Closer examination confirmed the differential effects as 25% of non-classical monocytes and less than 1% of classical or intermediate monocytes expressed sigma1 receptor. Finally, activation of the NR4A1 receptor, critical for non-classical monocyte survival and migration, successfully blocked immune infiltration following acute cocaine administration. Based on these experiments we hypothesize that rebalancing the immune profile may be an effective means to decrease immune infiltration into the brain in various pathologies including substance use disorder and addiction.

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T03 ADAPTIVE WORKING MEMORY TRAINING ON COGNITIVE PERFORMANCE IN HIV-1 PATIENTS

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Background: HIV patients often have working memory deficits, which may improve with working memory training (WMT). We investigated whether computer-based adaptive WMT may improve cognitive function on both near- and far-transfer cognitive tasks in HIV patients.
Methods: 116 adults were studied at baseline and 1-month after WMT. Participants were randomized to 25 adaptive or non-adaptive training sessions over 5-8 weeks (26 HIV+ adaptive, 33 HIV+ non-adaptive, 23 HIV- adaptive, 34 HIV- non-adaptive). Cognitive performance was assessed with 13 tests. Five assessed near effects: Symbol Span, Spatial Span, Spatial Addition, Digit Span, and Letter-Number Sequencing. Eight assessed far effects: Logical Memory, Designs, Letter Fluency, Color-Word Interference, Design Fluency, Trail Making, Rey Auditory Verbal Learning Test and Rey-Osterreith Complex Figure Test.
Results: Independent of serostatus, adaptive WMT led to greater improvement than non-adaptive WMT, with significant training-by-training type interactions in 3 of 5 near-transfer (p=0.002-0.029) and only 1 of 7 far-transfer tests (logical memory; p=0.004), after co-varying for age, education and general ability index. HIV+ subjects performed worse on verbal learning tasks, independent of training-type and training (p=0.004-0.048), but were comparable to controls on non-verbal learning tasks.
Conclusions: Regardless of serostatus, adaptive WMT led to greater improvements on untrained near transfer tasks compared to far transfer tasks. HIV+ participants had poorer cognitive ability on verbal learning independent of training.

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T04 Astrocyte-derived HIV evolution in humanized mice

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HIV infects the brain during acute infection and persists in the CNS despite suppressive antiretroviral therapy. While ample data demonstrates HIV genetic compartmentalization between the CNS and the periphery, it is unclear if HIV undergoes significant evolution within the CNS and whether CNS egress contributes to peripheral HIV quasispecies. We previously developed a humanized mouse model where HIV infected human fetal astrocytes were xenotransplanted into NSG mice and reconstituted with human PBMCs. Using this model, we demonstrated that astrocytes, the predominant brain cell type, support replication competent HIV which can egress from the CNS to peripheral organs (spleen, lymph node). In this study, we assessed genomic changes in HIV that egressed from astrocytes. Using single genome amplification and direct sequencing, the gold standard for HIV quasispecies characterization, we show that virus had evolved, with 24% of spleen sequences showing mutations from the

original viral inoculum. The mutated genomes showed random diversification, APOBEC mutations, Poisson distribution, star-like phylogeny, and time to most recent common ancestor analysis correctly identified the duration of infection (95% CI=27-61 days, actual=33 days), suggesting that viral evolution in this model fits the mathematical model of acute HIV-1 diversification in humans. Thus, astrocyte-derived HIV evolves and mimics the viral evolution observed in early peripheral infection and can contribute to peripheral genetic diversity. Ongoing studies are assessing HIV evolution within astrocytes over time.

T05 Stable histone modifications in post-mortem brain tissue can help overcome quality issues for identifying neuroHIV signatures

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Post-mortem human samples represent an extremely valuable resource with direct translational implications. Tissue repositories collect a great number of brain samples from HIV+ individuals and controls, at different ages and viral loads, treatments, substance use patterns and especially, different levels of cognitive functions. Yet, the use of many of these samples for the determination of global transcriptional changes, becomes impaired by problems with RNA quality. Post-mortem time length prior to tissue harvest is one of the causes of poor RNA quality. Histone modifications may be more stable than RNA in post mortem specimens, and may serve as surrogates of transcriptional patterns to help investigate global changes associated with disease. We hypothesized that post mortem stability may differ between epigenetic marks, and the most stable ones may be more consistently found in human post mortem samples with low RNA quality. For instance, H3K4me3 was detectable in post mortem tissues, both in mice and human specimens, and allowed the detection of inflammatory and viral correlates upon systems biology analysis approaches. Epigenetic strategies may allow retrieving perishable information from precious specimens, and further expanding the analysis of samples in the NNTC collection. This may help to increase the number of analyzed specimens, for improving statistical power while incorporating common confounders and heterogeneities of the human population.

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T06 Role of Microglia specific p38 signaling in NeuroAIDS

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HIV-1 causes severe and progressive neurocognitive impairment in humans. The HIV-1 envelope glycoprotein gp120 is an extracellular protein that is shed from infected cells and so has the potential to diffuse and interact with distant uninfected brain cells. Transgenic mice expressing HIV-1 coat glycoprotein gp120 in brain glial cells (HIVgp120tg) display neuropathological features similar to HIV dementia patients. During inflammation, p38 MAPK plays crucial roles in various pathological processes associated with HIV infection. Thus, p38 MAPK may be an important mediator in the development of HAND and immunodeficiency during HIV-1 infection. To determine the role of microglial p38 signaling in HIV mediated neuronal injury in vivo, we generated p38 floxed CX3CR1 Cre HIVgp120-expressing mice that have a p38 knockout specifically in microglia and macrophages. Immunofluorescence studies on brain sections of these mice were used to compare HIVgp120tg brains with and without microgliaspecific p38 knockout. Brain sections were stained for neuronal MAP2, presynaptic terminal protein synaptophysin and astrocytic glial fibrillary acidic protein, and nuclear DNA. Astrocytic GFAP levels remained unaffected by microglial p38 deficiency. However, we observed a significant loss of MAP2 and synaptophysin levels compared to non-tg controls only in HIVgp120tg animals with p38-expressing microglia but not in HIVgp120- expressing brains with microglia-specific p38 knockout. Thus, our model confirmed a critical role of microglial p38 MAPK in the HIV-1 neurotoxicity.

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T07 Gene editing strategies in the HIV-1 Tg rat brain: functional outcomes

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The HIV-1 transgenic (Tg) rat has been widely used to investigate the neurobiological basis of HIV-1 associated neurocognitive disorder (HAND) (McLaurin et al., 2018; McLaurin et al., 2017; Bertrand et al., 2018). Despite this wide experimental use of the HIV-1 Tg rat, several aspects of the HIV-1 Tg rat remain unspecified. We have investigated several key issues essential for gene excision studies: (1) HIV-1 proviral integration, (2) HIV-1 expression patterns in the brain, and (3) gene editing using CRISPR/Cas9 approaches and neurocognitive function. First, we used next-generation sequencing techniques and bioinformatics to detect insertion sites of the HIV-1 viral genome in the transgenic animals. We detected two independent chromosomal insertion sites with transgene concatemers, as the HIV-HIV junction patterns indicated multiple copies of HIV-1 DNA. Second, we used an ultra-sensitive mRNA in situ detection technology (Li et al., 2018) to determine the HIV-1 mRNA distribution pattern in the HIV-1 Tg rat CNS. We found that HIV-1 mRNA was differentially expressed by HIV-1 Tg rat brain regions. The highest gene expression was found in microglial cells of the mPFC; there was no expression in astrocytes. Finally, CRISPR/Cas9 HIV-1 mediated gene editing (Kaminski et al., 2016) of glial cells preserved the integrity and connectivity of neurons and improved neurocognitive function of HIV-1 Tg rats. In summary, these findings suggest the HIV-1 Tg rat is an important genetic model for critically testing gene excision approaches to removing the HIV-1 provirus from the brain.

T08* Epigenetic Therapy Approach in HIV-mediated Neurocognitive Impairment in Mice

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HIV infected individuals on antiretroviral therapy develop chronic mild neurocognitive impairment (NCI) that may affect everyday activities but do not progress to HIV dementia. The pathobiology of mild NCI is unclear and there is no treatment. We developed a chimeric HIV, EcoHIV, which can infect immunocompetent mice to investigate NCI biology and treatment. Infected animals develop chronic NCI that can be measured by behavioral tests and present transcriptional downregulation of pathways controlling learning and memory similar to gene expression profiles from patients with HIV cognitive disease. In order to analyze the epigenetic control of these functions we performed chromatin immunoprecipitation and next generation sequencing and found a high correlation between hypermethylation at histone 3 lysine 9 (H3K9) and transcriptional suppression of genes associated with synaptodendritic functions. To study the involvement of this change in NCI we used valproic acid (VPA) a histone deacetylase inhibitor that also decreases histone methylation at H3K9. VPA was administered daily one day

before EcoHIV infection or at different time points after infection on cognitive impaired mice. VPA treatment was able to both prevent and reverse NCI impairment, gene expression changes on key synaptic genes and histone hypermethylation on their promoters. The behavioral and molecular effects of VPA continued after VPA treatment discontinuation indicating a potential long term effect. The results suggest dysregulation of epigenetic control of memory in HIV induced mild cognitive dysfunction.

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T09 Membrane bound chemokines permit gesicle transduction in Jurkat cells

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Efficient vector delivery to target cells is continuously being pursued for effective gene-based therapies. Lentiviral vectors are often pseudo-typed with the vesicular stomatitis virus glycoprotein-G (VSV-G) which confers broad tropism due to its interaction with the widely expressed low density lipoprotein receptor (LDL-R) However, is has been shown that certain B cell and T cell populations are resistant to transduction by VSV-G pseudotyped vectors. Our lab has been working with VSV-G based microvesicles referred to as gesicles for the delivery of CRISPR/Cas9 ribonucleoprotein complexes. To enhance delivery to B and T cells, we tethered the chemokine ligands CCL2 or CCL5 to the membranes of HEK293FT producer cells. Chemokines are secreted proteins that play a multifunctional role in regulating cell migration, immunity, and development. Engineered gesicles expressing CCL2 or CCL5 and containing Cas9 targeted to the HIV long terminal repeat were used to treat an immortalized T-cell line, Jurkat-Tat that have been infected with the NLENG1 strain of HIV. We observed that only the gesicles created with chemokine ligands significantly decreased expression of p24 mRNA and protein. These data suggest that chemokines ligands may provide a method to target gene editing machinery to transduction-resistant cell populations.

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T10 HIV sensory neuropathy related behaviors in doxycycline inducible HIV-1 Tat transgenic mice

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HIV sensory neuropathy (SN) is frequently manifested with hard-to-manage pain and affects 30-50% of HIV/AIDS patients. Tat, transactivator of transcription, is a key activator of HIV transcription. However, the involvement of HIV Tat in neuropathic pain associated with HIV SN has not been extensively investigated. Here, we examined pain and functional related behavioral changes in doxycycline inducible HIV-1 Tat transgenic (iTat Tg) mice following the induction of Tat expression. Three induction regimens were tested: 7, 14 and 21-day daily injection of doxycycline (i.p. at 100mg/kg). Sensory sensitivities (mechanical, heat and cold) were assessed to evaluate pain-like behaviors. Sensory (pin prick assay) and motor (hind limb grip strength, toe spread reflex and toe spacing score) functional tests were used to evaluate hind paw functions associated with Tat expression. All regimens induced significant mechanical hypersensitivity and significant reduction of grip strength from within the first week post the initiation of Tat induction (day 0) to days 28 and 35 respectively in iTat Tg mice. Tat induction for 14 and 21 days also induced transient cold hypersensitivity in iTat Tg mice. None of these regimens induced significant heat hypersensitivity or affected other functional measurements. Together, our results suggest the utility of iTat Tg mice in studying the role of Tat in HIV SN. Sex-depended differences and Tat-induced molecular pathways will be further investigated in future studies.

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T11 HIV-1 Nef-EVs and morphine exert synergistic effects on alternative splicing of OPRM1 at both molecular and cellular levels

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Clinically used opioids, such as morphine, as well as illicit drugs of abuse, such as heroin, activate OPRM1, a member of the G protein-coupled receptor (GPCR) family. Examination of the opioid receptor genes showed that the OPRM1 gene undergoes extensive alternative splicing events. To date, 21 isoforms of the human OPRM1, with alternative C-terminal and/or N-terminal regions, have been identified. Given the importance of these regions in G protein-coupled receptor (GPCR) signaling, differential regulation of OPRM1 isoforms may have functional consequences. However, characterization of OPRM1 signaling is generalized, and only one isoform (MOR-1) has been extensively studied. Compounding this issue is the increasing significance of intravenous drug abuse in HIV neuropathogenesis. Multiple studies have shown that opioid abuse may potentiate HIV neurocognitive alterations indirectly by causing glial dysfunction or directly by stimulating overlapping signaling pathways in neurons. We recently reported that glial cells infected with HIV-1 release Nef protein in extracellular vesicles (Nef-EV). The Nef-EVs are readily taken up by neurons. Interestingly, Nef -EVs exert same effects as those with morphine on alternative splicing of OPRM1 pre-mRNA by inducing the alternative splicing of MOR-1X isoform. These suggest that Nef-EVs might exert action on OPRM1 as morphine does to initiate OPRM1-mediated signal transduction. More interestingly, combined treatment with Nef-EVs and morphine has a synergistic effect on alternative splicing of MOR-1X isoform in both primary human and rat neurons. Furthermore, the impact of morphine on expression of key genes involved in opioid dependence was modulated by Nef-EVs in neuronal cells. Nef-EVs and morphine exert synergistic effects at both molecular and cellular levels and these studies suggest that actions of Nef-EVs and morphine could also converge at the behavior level.

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T12 Chronic treatment of Triumeq induces neuronal hyperactivity and over-activation of voltage-gated ca2+ channels in the medial prefrontal cortex of rats

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Combined antiretroviral therapy (cART) suppresses HIV replication, improves immune function, and prolongs life of HIV+ patients. Despite cART, the prevalence of HIV-associated neurocognitive disorders (HAND) occurs in ~50% of HIV+ patients. It is unknown if the neurotoxicity side effects of cART worsen HIV dysregulation in the medial prefrontal cortex (mPFC), which regulates neurocognition. We have shown that lamivudine (3TC), a nucleoside reverse transcriptase inhibitor (NRTI), increases firing and voltage-gated Ca2+ channel (VGCC) activity in mPFC

pyramidal neurons. However, the chronic effects of cART is unknown. Triumeq is a first-line cART regimen for treating HIV/AIDS. It is formulated by abacavir (ABC, a NRTI), dolutegravir (DTG, an integrase inhibitor), and 3TC (a NRTI). Here we assessed the acute (in vitro) and chronic (in vivo) effects of Triumeq on activity of mPFC neurons in rat brain slices using whole-cell patch-clamp recording. We found that at a concentration similar to that detected in the cerebrospinal fluid (CSF) of HIV+ patients, acute Triumeq did not affect firing; but at 10x or 100x higher concentrations, it significantly increased firing. Further, a 4-week (but not 2-week) chronic Triumeq treatment (s.c.) significantly increased firing, which was associated with enhanced Ca2+ influx via VGCCs. These novel findings demonstrate that chronic Triumeq treatment increases mPFC neuron activity by enhancing Ca2+ influx via over-activated VGCCs, suggesting that such side effects of cART may exacerbate HIV-induced neurotoxicity in the mPFC during aging.

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T13* Combinatorial effects of HIV Tat and cocaine-mediated activation of microglial NLRP3: Implications for NeuroHIV

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Abuse of cocaine in the context of HIV-1 infection potentiates neuroinflammation that underlies HIV-1-associated neurocognitive disorders. Although emerging evidence indicates the involvement of NLRP3 in HIV and cocaine-mediated glial activation and neuroinflammation, mechanisms underlying this process remain less clear. Since HIV Tat has been shown to upregulate the expression of NLRP3 and cocaine is a known inducer of reactive oxygen species, we hypothesized that exposure of microglia to Tat and cocaine could lead to exaggerated activation of the NLRP3 inflammasome. To address this, mouse primary microglia (mPm) were exposed to Tat &/or cocaine or left untreated and evaluated for the expression of NLRP3. Exposure of mPm to both Tat and cocaine resulted in upregulated expression of NLRP3 compared to cells exposed to either agent alone. Exposure of microglia to both agents also increased the expression of mature IL-1 beta suggesting increased activation of NLRP3 by these agents. Gene-silencing of NLRP3 (siNLRP3) attenuated IL-1 beta release thereby underscoring the role of NLRP3 in Tat & cocaine-mediated activation of microglia. Collectively, these findings suggest that both Tat & cocaine could, via their co-operative actions, exacerbate microglial activation, thereby compounding the severity of HIV and cocaine-induced neuroinflammation. The future directions of this study involve exploring the potential of NLRP3 inhibition in alleviating neuroinflammation in the context of co-morbid chronic viral infections and drug abuse of cocaine.

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T14 17 alpha-Estradiol is a protective factor in HIV-1 gp120 induced neuronal dystrophy

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Estrogen deficiency has been implicated as a risk factor for the development neurodegenerative diseases including HIV-1 associated neurocognitive disorder and Alzheimer's disease, and estrogen treatment offers neuroprotection against the cognitive decline. Recent studies indicate that cognitive function is related to estrogen receptor-alpha(ERα); however, the underlying mechanism is not well understood. In primary cultured hippocampal neurons, we found that ERalpha localized to LAMP1 positive vesicles, and that 17alpha-estradiol, but not 17beta-estradiol acidified endolysosome pH. Consistent with these observations, 17alpha-estradiol increased the numbers of active cathepsin D positive vesicles. Thus, we evaluated the effect of 17alpha-estradiol in HIV-1 gp120-induced neurodegeneration. We demonstrated that gp120 de-acidified endolysosome pH and treatment of primary rat hippocampal neurons with HIV-1 gp120 induced swellings throughout the length of the neurites, which were characterized by the accumulation of LAMP1 positive vesicles. These LAMP1 positive neurite swelling co-existed with an increased abundance of LAMP1 vesicles throughout the neurons. Pre-treatment with 17alpha-estradiol showed a reduction in the number as well as the size of these LAMP1 positive neurite swelling. Our findings suggest that 17α-estradiol is protective against gp120-induced neuronal dystrophy. Further understanding the role of 17alpha-estradiol in endolysosome function and trafficking could lead to the development of novel therapeutic strategies against neurodegeneration associated with HIV.

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T15 Reverse Transcriptase Inhibitors Alter Mitochondrial Biogenesis and Inflammation in Neurons and Glia

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Mounting evidence suggests that ART drugs may contribute to the prevalence of HIV-associated neurological dysfunction. Alterations in mitochondrial function and increased inflammatory gene expression are common pathological findings in tissues from decedents that were diagnosed with HAND and HIV-SN and on ART. In this study, we hypothesized that ART drugs contribute to neurotoxicity by modulating mitochondrial biogenesis and inflammatory pathways. Primary human astroglia and human neuroblastoma (SH-SY5Y) cells were exposed to Tenofovir (TDF), Efavirenz (EFV), Ritonavir, or Dolutegravir for 24 hours and then analyzed for mitochondrial alterations and inflammatory gene expression. The reverse transcriptase inhibitors (RTI), TDF and EFV, showed the most robust alterations in transcription factors that regulate mitochondrial biogenesis (PGC1α and TFAM), as well as alterations in mitochondrial activity. The RTI drugs also induced inflammatory responses from the astroglia; however, astroglial responses to the RTIs were not consistent among different donors, suggesting that heterogeneity may contribute to variable responses to ART drugs. To determine if RTIs affect mitochondrial biogenesis and gliosis in vivo, TDF was administered to wild-type (wt) mice and the GFAP-gp120 transgenic (tg) mouse model. In the wt and tg mice, TDF increased expression of GFAP, decreased expression of IBA1 and altered expression of TFAM in neurons and astroglia. These data suggest that RTIs may contribute to alterations in mitochondrial activity and inflammatory signaling in HIV+ persons on ART.

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T16 Role of purines and purinergic receptors in NeuroHIV

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Anti-retroviral therapeutics (ART) made human immunodeficiency virus (HIV-1) a manageable, chronic disease. Despite the efficacy of ART, at least half of the HIV-positive population develops HIV-1 associated

neurocognitive disorder (HAND) due to chronic neuroinflammation. Our data indicate that circulating level of ATP can be a potential biomarker of HAND in the HIV-infected population. A potential source of extracellular ATP is the opening of pannexin-1 channels (Panx-1), which normally closed under healthy conditions. We found that Panx-1 channels are open in PBMC isolated from HIV-infected individuals, which correlates with increased serum and plasma levels of ATP and PGE2. We demonstrated also that high circulating ATP level contributes to blood-brain barrier (BBB) compromise and transmigration of HIV-infected PBMC in response to CCL2 into the brain. Thus, increased levels of ATP can disrupt the BBB by purinergic-related mechanisms. To address this, we measured expression levels of ATP/ADP/AMP/ADN receptors as well as ecto-ATPases in human BMVEC and astrocytes. Normally, extracellular ATP activates purinergic receptors and is degraded to ADP and AMP via E-NTDPase and by Ecto-5'-NT/CD73 to ADN. Currently, we are measuring levels of purine receptors and enzymes in control and HIV-infected cells. We are also determining regulation of connexin 43 (Cx43) by ATP in the context of HIV infection. Our results indicate that blocking purinergic receptor activation could prevent the increased BBB permeability the occurs as a result of HIV-1 related inflammation in the brain.

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T17 Role of differential packaging within Tobacco/HIV-induced exosomes from macrophages in HIV pathogenesis

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Tobacco smoking is highly prevalent in HIV patients. Enzymes metabolize cigarette smoke constituents (CSC), causing oxidative stress and cytotoxicity. However, the mechanism behind tobacco-induced, oxidative stress-mediated HIV replication is still unknown. Exosomes have recently gained attention for their unique nature as intercellular vehicles, which can package proteins, mRNA, cytokines, miRNA, etc. We hypothesize that tobacco and/or HIV exposed monocyte-derived exosomes and their components, especially pro-oxidant factors, are key mediators for HIV replication. Previously, we demonstrated that CSC-induced exosomes have altered antioxidant contents, which protect against toxicity and viral replication in early stages of infection. This led us to investigate differential packaging of specific contents in exosomes subjected to CSC and HIV exposure. Pro-inflammatory cytokines, especially IL-6 and IL-8, are highly packaged in CSC- and HIV-induced exosomes. Interestingly, anti-inflammatory cytokines, particularly IL-10, have lower rates of exosomal packaging while chemokines are mostly increased. Our in vitro findings are similar to ex vivo data, which suggests that smoking and HIV induce inflammation by altering cytokine levels in exosomes, which may explain immunologic responses in HIV-smokers. Further, we will investigate components, like benzopyrene and nicotine within CSC, which alter packaging in the exosomes, and their underlying mechanism, to establish a mechanistic pathway of tobacco/HIV-induced impacts on HIV.

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T18 CRISPR/Cas-9 Oxytocin Nanodelivery: A New Approach to Enhance BBB Transport and Endogenous Expression in Drug Abuse

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Drug abuse is associated with serious medical and health consequences including dysregulation of the endocrine-metabolic system and neurobehavioral alternations. It has been shown that endogenous Oxytocin (OT) delivery inhibits the development of acute/chronic morphine tolerance and attenuate the symptoms of morphine withdrawal in a dose-dependent manner. Repeated morphine ingestion upregulates opioid receptor and leads to suppression of OT production, which eventually causes the development of tolerance and physical dependence. The aim of this work was to develop the OT-CRISPR_Cas-9/gRNA nanoformulation (NF) to treat the morphine abuse in HIV-1 infection. The NF was prepared by simultaneous spray (SS) novel technique using lab developed non-viral transfecting reagent P(SiDAAr)5P3. The NF was prepared and characterized for cell uptake, cytotoxicity and NF efficacy for OT production using primary human neurons on exposure to Morphine ± HIV-1. Results showed high cell uptake of NF, good transfection efficiency for P(SiDAAr)5P3 (>70 ±12%) NF and lower cytotoxicity (>92%) in comparison to standard jetPEI polyplex NF (> 68%) in primary human neurons. Also, the NF treatment able to produce a measurable amount of OT after 48 hrs of treatment (ELISA assay) in Morphine ± HIV-1 treated cell culture model. Currently, we are testing the aerosol-based NF via intranasal delivery and its efficacy using OT deficient mice model.

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T19 Drug use is associated with anti-CD4 IgG-mediated CD4+ T cell death and poor CD4+ T cell recovery in viral-suppressive HIV-infected individuals under antiretroviral therapy

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Background. The role and mechanism of drug use or abuse in antiretroviral therapy (ART)-treated HIV disease are not completely known. Methods. To investigate the impact of drug use on HIV pathogenesis without confounding by HIV replication and ART adherence, we first analyzed the data from our clinical database in 103 HIV+ subjects with viral-suppressed ART by a multiple regression test. Results. We found that HIV+ drug users had lower CD4+ T cell counts but higher CD8+ T cell counts compared to HIV+ non-drug users (Mann-Whitney, $P< 0.05$). Plasma anti-CD4 IgG level was associated with increased plasma IL-23 level ($P<0.05$). Conclusion: These results suggest that drug use prevents immune reconstitution in HIV-infected individuals despite long-term ART and viral suppression.

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T20 BK channels regulate extracellular Tat-mediated HIV-1 LTR transactivation

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HIV-1 Tat is essential for HIV-1 replication and plays an important role in the NeuroHIV complications like HIV-1 associated neurocognitive disorders (HAND). Secreted from infected or transfected cells, Tat can be taken up by all CNS cells via receptor-mediated endocytosis. Upon endocytosis, Tat is internalized into endolysosomes, from which it escapes into the cytoplasm before entering the nucleus to facilitate HIV-1 viral replication. It is well known that neutralizing the acidic pH of endolysosomes with chloroquine prevents exogenous Tat degradation and enhances the bioavailability of Tat to activate HIV-1 LTR in nucleus. Thus, acidifying endolysosomes could restrict extracellular Tat-mediated HIV-1 LTR transactivation. In the present studies, we determined the involvement of TRPML1 and BK channels in regulating endolysosome pH, as well as Tat-mediated HIV-1 LTR transactivation in U87MG cells stably integrated with HIV-1 LTR luciferase reporter. We demonstrated that activating TRPML1 channels with ML-SA1 acidified endolysosomes and restricted Tat-mediated HIV-1 LTR transactivation, and these beneficial effects of ML-SA1 are

dependent on subsequent BK-channels, as evidenced by findings that pharmacological blocking or shRNA knocking down of BK channels blocked ML-SA1's effect in restricting Tat-mediated HIV-1 LTR transactivation. In conclusion, acidifying endolysosomes by activating TRPML1 and BK channels can be a therapeutic strategy against latent HIV-1 infection, HIV-1 associated neurocognitive disorders, and other HIV-1 comorbidities.

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T21 Differential Regulation of TREM2 and CSF1R in CNS Macrophages in the SIV/Macaque Model

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HIV-induced CNS disease, like many neuroinflammatory conditions, has been shown to be primarily driven by CNS macrophages, including microglia; however, regulation of CNS macrophage immune responses remain incompletely understood. TREM2 (triggering receptor expressed on myeloid cells) is constitutively expressed by microglia and contributes to cell survival, proliferation, and differentiation. Non-functional mutations in TREM2 are known risk factors for neurodegenerative diseases including Alzheimer's and ALS; recent reports also have indicated a role for TREM2 in HIV. Using in situ hybridization (ISH) and qRT-PCR in an SIV/macaque model of HIV CNS disease, we found that TREM2 mRNA levels were significantly elevated in the frontal cortex of animals with SIV encephalitis ($P = 0.02$) . Despite mRNA upregulation, TREM2 protein was not significantly elevated when measured by ELISA. Previously, we characterized the expression of CSF1R (colony stimulating factor 1 receptor) in this model. TREM2 and CSF1R share the same promoter, PU.1. While TREM2 and CSF1R mRNA levels in frontal cortex were highly correlated (Spearman $R = 0.79$, $P < 0.001$), protein levels were not well correlated. In SIV-infected macaques released from cART to study viral rebound, neither TREM2 nor CSF1R mRNA increased with rebound viremia. However, the fold increase in CSF1R protein was significantly higher than TREM2 ($P = 0.02$). This differential expression suggests TREM2 and CSF1R play unique roles in the pathogenesis of HIV CNS disease despite sharing the same promoter, likely mediated post-transcription.

T22 Proteomic and cytokine profiling of exosomes derived from plasma of HIV-infected alcohol drinkers and cigarette smokers.

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Background: Abuse of alcohol and tobacco can enhance HIV pathogenesis. Exosomes present a stable and accessible source of biological information from one cell to various types of cells. Therefore, we aimed to study the specific exosomal proteins, cytokines and chemokines which are altered in both HIV and drug abusers to identify a physiological marker to indicate the immune status and neuronal damage of HIV-positive drug abusers. Methods: Exosomes were isolated from plasma samples of the following subjects: a) Healthy b) HIV c) Alcohol drinkers d) Smokers e) HIV+drinkers f) HIV+ smokers. Quantitative proteomic and cytokines profiling were performed. Results: A total of 343 proteins were detected in exosomes of all study groups. Comparison of proteins among groups revealed that hemopexin was not significantly altered in drinkers or HIV patients, but was decreased in HIV+drinkers. Further, our study is the first to show properdin expression in plasma exosomes, which we found was decreased in HIV+smokers and HIV+drinkers compared to HIV non-abusers. Plasma exosomes package cytokines and their levels are altered in HIV-positive drug abusers. The percentages of exosomal IL-1ra and RANTES were significantly reduced in HIV patients compared to healthy subjects. Conclusion: The present findings suggest that hemopexin and properdin show potential as markers for HIV-drug abuse interactions. Further, altered cytokine levels in the plasma, as well as exosomes of HIV-positive drug abusers, suggest a novel mechanism of neuropathogenesis in cases of drug abuse and HIV comorbidity.

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T23 Cecal ligature and puncture-induced systemic inflammation increases water diffusion in white matter-rich regions in the absence of blood-brain barrier breakdown: relationships to changes in glial cell morphology

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Blood-brain barrier breakdown (BBB) has been proposed to underlie sepsis-associated encephalopathy (SAE). Cecal ligature and puncture (CLP) in rats induced deficits in the righting reflex and resulted in higher T2-weighted contrast intensities in the cortex, decreased blood perfusion distribution to the cortex and increased water diffusion anisotropy in the corpus callosum. CLP also reduced the number of fragments staining for microglia- and astrocytic-specific proteins but did not induce widespread perivascular IgG diffusion. In conclusion, experimental SAE can occur in the absence of BBB breakdown and is accompanied by changes in water diffusion and glia cell morphology in brain white matter.

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T24 EXPRESSION OF CYP2E1 IN PLASMA EXOSOMES AND ITS CRITICAL ROLE IN DRUG-INDUCED TOXICITY

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The presence and functional role of the major drug metabolizing cytochrome P450 (CYP) enzymes in human plasma exosomes unknown. We studied the functionality of CYP enzymes, especially CYP2E1, in human plasma exosomes. We further studied the effect of plasma exosomal CYP2E1 in mediating ethanol (ETH) and acetaminophen (APAP) induced toxicity. First, we isolated exosomes from plasma obtained from de-identified healthy individuals and two clinically relevant cell lines-hepatic and monocytic cells. Upon characterizing the exosomes for their physico- and biochemical properties, we measured the contribution of these exosomal CYP2E1 in mediating cytotoxicity. We observed that the relative level of CYP enzymes in exosomes is higher than in plasma, suggesting their specific packaging in exosomes. Interestingly, the relative level of CYP2E1 mRNA was much higher than others, and showed

substantial enzymatic activity. We also found that CYP2E1 is expressed relatively higher in plasma exosomes than exosomes derived from hepatic and monocytic cells. We observed that the plasma exosomal CYP2E1 cargo played a synergistic role in mediating ETH and APAP induced toxicity in a time dependent manner in primary hepatocytes and monocytes. Finally, we determined the effect of alcohol-drinking on the level of plasma exosomal CYP2E1 in an animal model, and the effect of this CYP2E1 on ethanol-induced toxicity in hepatic cells. This is the first evidence of the substantial expression and circulation of CYP2E1 in plasma exosomes and their crucial role in mediating drug-induced toxicity.

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T25 Prolonged exposure to HIV-1 Tat or acute morphine differentially alter firing rates in dopamine D1 and D2 receptor-expressing medium spiny neurons in the striatum.

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Despite the advent of combination anti-retroviral therapies, 30-50% of HIV-infected individuals still exhibit neurocognitive disorders. The striatum appears to be especially vulnerable to HIV-1 infection, harboring high viral loads and exhibiting medium spiny neuron (MSN) damageâ €"presumably leading to motor deficits that can occur with neuroHIV. Synaptodendritic injury in MSNs can be modeled with HIV-1 Tat exposure and exacerbated by chronic co-exposure to opiates. Because HIV-infected individuals can develop intractable painful neuropathy that is often treated acutely with opiates, we assessed the pathophysiological effects of acute opiate exposure in vulnerable neuronal populations. To discern the effects of Tat and opiates on the main MSN types, we crossed Drd1-tdTomato or Drd2-eGFP lines with conditional HIV-1 Tat transgenic mice. Whole-cell patch clamp recordings of MSNs were performed in ex vivo striatal slices to explore progressive deficits caused by Tat. Our data indicate that 48 h or 2-weeks Tat exposure significantly increased D2 MSN firing rates. By contrast, D1 MSNs showed initial 48 h increases, followed by a transient decrease (2 weeks), in firing rates in response to Tatâ€"suggesting that D1 MSNs are highly plastic and uniquely vulnerable to Tat depending on the duration of exposure. Acute morphine exposure increased D1 MSN firing rates irrespective of Tat and variably affected the response of D2 MSNs. Because relatively few MSNs express mu opioid receptors this suggests that morphine's effects are independently mediated by glia or interneurons.

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T26 Restricted and Region-specific HIV-1 mRNA expression in the HIV-1 Transgenic rat brain: role for microglia, but not astrocytes

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The HIV-1 Transgenic (Tg) rat has been widely used to study HAND. However, the actual HIV-1 mRNA neuroanatomical expression pattern in the HIV-1 Tg rat brain is still unknown. In the present study, we performed a highly sensitive RNA in situ detection technology (RNAscope) to observe the HIV-1 mRNA distribution in specific neuroanatomical brain regions. Furthermore, we used specific cell-type markers of Iba1 and GFAP to dual-label the microglia and astrocytes, respectively, together with a HIV-1 RNA probe to verify the major cell type of HIV-1 expression in HIV-1 Tg rats. Our data indicated a highly restricted HIV-1 mRNA distribution pattern in the HIV-1 Tg rat brain. Specifically, the most abundant HIV-1 mRNA expression was located in the cerebral cortex. The medial prefrontal cortex had high level of HIV-1 mRNA expression, whereas the hippocampus had a relatively low level of expression. The dual-labeling results show that microglia appeared to be a major cell type; astrocytes had little, if any expression. Furthermore, primary cultured microglia purified from mixed glia cells (verified by cell marker: Iba1, MOMA and CD11b) from the HIV-1 Tg rat showed a high level of HIV-1 mRNA expression. In summary, although the HIV-1 provirus is located in all cells, the prefrontal cortex region and microglia actively express HIV-1 mRNAs. Identifying the region-specific and cell-specific expression of HIV mRNA in the HIV-1 Tg rat offers valuable insight for study HIV-1 associated neurocognitive disorders.

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T27 Impact of fingolimod (FTY720) on acute inflammation and gliosis in chronic constriction injury

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Fingolimod (FTY720), a sphingosine-1-phosphate (S1P) receptor modulator, has been shown to reduce allodynia and hyperalgesia within rodent neuropathic pain models. Potential therapeutic mechanisms for FTY720 include modulation of afferent pain signaling pathways, and suppression of localized inflammation along these pathways. Using a chronic constriction injury (CCI) mouse model, we studied the impact of FTY720 administration on proinflammatory cytokine production and gliosis within the lumbar spinal cord and sciatic nerve of male C57BL/6J mice (n=6-12, 8 wks old). Within sciatic nerve, CCI increased expression of TNF-alpha, CCL3, and CCL5 at 8 d post-surgery. CCL3 was likewise elevated in lumbar spinal cord. A single injection of FTY720 (3 mg/kg, i.p.) 24 h prior significantly reduced mechanical allodynia and reduced concentrations of proinflammatory chemokine CCL5 at the site of injury from 15.18 +/- 1.61 to 6.36 +/- 0.76 pg/mL (mean +/- SEM, $p < 0.001$), with similar trends in CCL3 and TNF-alpha. Within lumbar dorsal horn, CCI increased the density of Iba1+ microglia from 5.56 +/- 0.53 cells/mm^2 to 13.21 +/- 1.67 cells/mm^2 ($p < 0.001$) by 9 d post-surgery. However, repeated dosing of FTY720 (q.d., d 7-9) did not alter the density of microglia or astrocytes within dorsal horn, despite significantly reduced mechanical allodynia. While FTY720 treatment did not impact spinal glial density acutely, the reduction in proinflammatory cytokines at the sciatic nerve likely contributes to the long-term antinociceptive impact of FTY720 in CCI.

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T28* Microglia-mediated neuroinflammation bidirectionally modulates morphine reward

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Neuroinflammation has been shown to paradoxically both reduce and increase the salience reward of abused substances like morphine. The mechanisms underlying this dynamic multiphasic modulation are poorly

understood. We hypothesize that microglia-mediated neuroinflammation mediates neurochemical changes in reward circuitry, thereby dynamically modulating the rewarding effects of morphine. Testing this, C57BL/6J mice were administered i.c.v. LPS to evoke an episode of controlled and brain-selective neuroinflammation. LPS-induced neuroinflammation produces a dynamic and multiphasic modulation of reward, both suppressing and potentiating morphine-conditioned place preference (CPP) and voluntary consumption in a two-bottle choice (TBC) assay in a time-dependent manner. The LPS-induced modulation of morphine-CPP effects were mimicked by cytokine IL-1β administration, but prevented by pretreatment with the anti-inflammatory indomethacin (10 mg/kg, i.p.), and absent in Secreted Phosphoprotein 1 gene-disrupted (Spp1 KO) mice known to possess impaired microglial responses. Work is underway to examine modulatory contributions of microglial activation on reward, using cells isolated from brains of both wild type and Spp1 KO mice to characterize the basal and LPS-stimulated neuroinflammatory status along with measured expression of key inflammatory markers with immunocytochemical and biochemical analysis. Collectively, these results affirm neuroimmune status modulates the behavioral motivation for rewarding substances while establishing correlations with microglial activity status.

T29 Interactions Between HIV and Aging on the Neural Dynamics Underlying Selective Attention

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Background: HIV is associated with age-related comorbidities and neurocognitive deficits, leading to hypotheses of HIV-related premature aging. Selective attention deficits and related neural dynamics have been associated with HIV-associated neurocognitive disorder, however the additive effect of aging on this process is not understood. Therefore this study aimed to identify the interactive effects of aging and HIV on selective attention processing. Methods: 166 participants (92 control, 72 participants with HIV) performed a visual selective attention task while undergoing magnetoencephalography (MEG). Frequency-specific neural responses related to selective attention were identified and imaged. Reaction time and neocortical activity were analyzed with ANCOVAs aimed at examining the age by HIV interaction term. Results: Reaction time analyses showed a significant interaction such that older age was associated with increased selective attention interference in controls, and selective attention deficits were present in HIV regardless of age. Significant theta-frequency activity was imaged, and age by HIV interaction effects were found in the middle frontal gyrus and posterior parietal cortex. Conclusions: Behavioral selective attention performance in participants with HIV was near the level of older controls across all ages, providing evidence of HIV-related premature aging. MEG indicated probable compensatory activity in posterior parietal and middle frontal cortex as participants with HIV age. These findings highlight the divergent aging trajectory of people living with HIV

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T30 Alterations in amyloid precursor protein secretases in a model of HIV-induced neurotoxicity

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Aging is increasingly appreciated as a contributor to HIV-associated neurocognitive disorders (HAND) since antiretroviral therapy has increased the life expectancy of patients. Accumulation of extracellular plaques composed of amyloid-beta (A-beta) is observed in aging-related neurodegenerative disorders. A-beta is produced by the cleavage of amyloid precursor protein (APP) by BACE1, but is precluded by ADAM10 cleavage. A-beta accumulation and increased BACE1 levels have been observed in models of HAND, suggesting altered APP processing as a potential contributor to HIV-mediated neurotoxicity. In models of Alzheimer's Disease, BACE1 up-regulation was shown to be mediated by the unfolded protein response kinase PERK. Therefore to characterize APP processing and its regulatory mechanisms in the context of HIV, primary rat cortical neurons were treated with HIV-infected monocyte-derived macrophages supernatants (HIV/MDMs), NMDA or a PERK activator (PA) with or without 1hr PERK inhibitor pre-treatment. Western blotting and MAP2 staining were performed to determine protein levels and neurotoxicity levels, respectively. Only PA treatment led to an increase in BACE1 levels when compared with untreated cultures. Further, HIV/MDMs, NMDA and PA treatment led to a decrease in ADAM10 levels when compared with untreated cultures, suggesting that due to a reduction in its non-amyloidogenic processing, altered APP processing might be occurring in HIV and HIV-related insults. Future studies will delve into this process by assessing products of APP cleavage such as A-beta in our model.

T31* Neuroprotective and anti-inflammatory properties of the neurotrophin receptor ligand, LM11A-31, in animal models of HIV neuropathogenesis

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The neurotropic, pro-survival actions of mature neurotrophins offer enormous therapeutic potential for treatment of neurodegenerative diseases. These beneficial actions contrast with the immature pro-neurotrophins which increase with age, disease or damage and facilitate neurodegeneration via high affinity interactions with the p75 neurotrophin receptor (p75NTR). In gp120 Tg mice, we observed an age dependent accumulation of p75NTR that paralleled microglial activation, MAP-2 loss and accumulation of Tau-like oligomeric clusters in the hippocampus. Treatment with the small, non-peptide ligand, LM11A-31, designed to reestablish a favorable balance of neurotrophic activity, reduced microglial activation and restored MAP-2 expression in the hippocampus. Accumulation of the p75NTR and Tau clusters was unaffected except in the oldest mice where the drug prevented a natural decline in Tau clusters. In separate experiments, cats infected intracranially with FIV were treated with 13 mg/kg LM11A-31 for 10 weeks beginning at 17.5 months to evaluate efficacy in animals with active viral infection. A small reduction in CSF FIV and an increase in the CD4:CD8 ratio were seen with no adverse effects on daily activities, blood chemistry and CBC. Behavioral assessments beginning at 18 months post-inoculation, where cognitive deficits begin to appear, showed improved performance in a modified T maze and less anxious behavior in an open field. Thus, LM11A-31 was safe and effective in preventing pathology and cognitive decline with no adverse effects on physiology or viral synthesis.

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T32 Identifying a role for E2F1 in synaptodendritic damage in mouse models of HIV

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E2F1 is a transcription factor important for G1-S phase cell cycle transition that has the potential to contribute to the development of HIV-associated neurocognitive disorders (HAND). In the CNS of HIV-infected patients with encephalitis E2F1 exhibits altered expression, and in rodent models loss of normal E2F1 function impairs synaptic health, learning and memory. To study the relationship between E2F1 and HIV-related neuropathogenesis, we used two different HIV-related mouse models that exhibit neurocognitive impairment: brain-specific GFAP-driven Tat-inducible transgenic (GT-tg) and mouse-tropic HIV infection (EcoHIV) which utilizes a chimeric HIV that expresses ectopic murine leukemia viral envelope instead of HIV's normal envelope protein, gp120. Adult male GT-tg mice received saline or doxycycline (100mg/kg/day, i.p) for seven days to induce Tat expression or adult male wildtype mice were inoculated with EcoHIV (i.p). GT-Tg mice were euthanized 1 day after treatment and EcoHIV mice were euthanized 4-week post inoculation. Brain tissue was collected for quantification of E2F1, cleaved alpha-spectrin, and post-synaptic proteins by western blot to identify potential mechanisms of synaptodendritic damage. In both models E2F1 expression was increased, which correlated with increased cleaved alpha-spectrin in GT-Tg mice and reduced PSD-95 expression in EcoHIV-infected mice. These results support our hypothesis that E2F1 plays a role in either the development or persistence of neurocognitive deficits in HIV-infected patients.

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T33 A solution at HAND: Glycogen Synthase Kinase-3 inhibition rescues neurocognitive deficits in a murine model of HIV-Associated Neurocognitive Disorder

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A high percentage of patients with HIV on antiretroviral therapy (ART) present with HIV-associated neurocognitive disorders (HAND). The severity of HAND symptoms can increase as patients on ART live longer. There is a critical need to develop novel treatments for HAND. This study proposes a new murine model and a potential therapy for HAND. Mimicking HIV-infected patients on ART, HIV-1-transgenic 26 (Tg26) mice serve as a model by expressing low brain levels of viral peptides in the absence of active viral replication. Glycogen synthase kinase-3 (GSK-3), a serine/threonine protein kinase, was investigated as a therapeutic target to restore cognition in this mouse model. Experiments were done to assess learning and memory in Tg26 mice and their responses were compared to the wild-type (WT) littermates. Hippocampal electrophysiology (long-term potentiation; LTP) was performed in organotypic slices. We observed sex-dependent deficits in spatial- and contextual-memories in male Tg26, but not in female mice. Consistent with the cognitive deficits, male Tg26 showed a suppression of hippocampal LTP in area CA1, and reduced synapsin-1 and BDNF levels compared to WT controls. Importantly, as a potential treatment, pharmacological inhibition of GSK-3 restored hippocampal LTP and cognition, consistent with induction of synapsin-1 and BDNF levels. In summary, we provide evidence that Tg26 mice are suitable for HAND research, and that GSK-3 is a promising therapeutic target in HAND. Furthermore, this study stresses on the importance of incorporating sex as a biological variable in HAND.

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T34 HIV-1 Tat systematically disrupts cortico-basal ganglia-thalamo-cortical synaptic circuitry and associated behaviors in transgenic mice

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Many HIV-1-infected individuals experience neuropsychological deficits in executive function despite antiretroviral therapy. HIV-1 Tat disrupts neuronal morphology and function causing neurocognitive deficits. Although the striatum has been shown to be particularly vulnerable to HIV-1 Tat damage, there is a paucity of research on how these deficits might result in systems-wide, pathophysiological alterations in cortico-basal ganglia-thalamo-cortical circuitry. Accordingly, we investigated the effects of 8 weeks HIV-1 Tat induction on neuronal synaptic connectivity and associated behaviors within the anterior cingulate cortex (ACC), striatum, and mediodorsal (MD) thalamus in adult male transgenic mice. In the ACC, HIV-1 Tat decreased the colocalization of inhibitory pre- and post-synaptic proteins synaptotagmin 2 and gephyrin, respectively, but did not affect the density of layer V pyramidal cell dendritic spines, indicating that in the ACC HIV-1 Tat preferentially interferes with inhibitory synapses. Similarly, HIV-1 Tat did not affect spine density in the MD thalamus. However, supporting previous data, HIV-1 Tat decreased spine density in the striatum. The synaptic changes coincided with HIV-1 Tat increasing novelty seeking, while decreasing prepulse inhibition, indicating that HIV-1 Tat may cause impulsivity and attention deficits. Together, these data suggest that HIV-1 Tat uniquely and systematically disrupts inhibitory and excitatory synapses throughout the cortico-basal ganglia-thalamo-cortical circuit and mediates changes in impulsivity and attention.

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T35 Cocaine activates microglia via miR-148b-lncRNA XIST-DNMT1 axis-mediated downregulation of an anti-inflammatory gene, PPARG

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Cocaine, one of most commonly abused drugs, has been shown to activate microglia both in vitro and in vivo. However, the detailed epigenetic and molecular mechanism(s) underlying cocaine-mediated microglial activation remain poorly understood. It is well-known that cocaine can modulate the levels of targeted genes through the epigenetic mechanism(s) via DNA methylation, histone modifications and non-coding RNAs. In this study, we tested the hypothesis that exposure of mouse microglial cells to cocaine activates microglia involving downregulation of the peroxisome proliferator-activated receptor gamma (PPARG) via the miR-148b-DNMT1-lncRNA XIST axis. We performed RT2 lncRNA PCR Array Mouse IncFinder on cocaine-exposed microglial cells that demonstrated increased expression of lncRNA XIST compared with control. qPCR analysis further validated the increased expression of lncRNA XIST in cocaine-exposed microglial cells. Bioinformatics

analysis, miR target validation and RNA immunoprecipitation assays suggested the possible binding of lncRNA XIST with miR-148b and DNMT1, thereby leading to increased expression of DNMT1 in cocaine-exposed microglial cells. Overexpression and gene-silencing approaches were employed to validate the involvement of miR-148b-lncRNA XIST-DNMT1 signaling axis in negative regulation of PPARG-mediated activation of microglia in the presence of cocaine. These findings demonstrated the role of miR-148b-lncRNA XIST-DNMT1 axis in downregulation of PPARG, leading in turn, to cocaine-mediated activation of microglia.

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T36 HIV Tat-mediated astrocyte senescence involves lncRNA TUG1-SIRT1 axis: Implications for HIV induced accelerated aging

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With the success of combined antiretroviral therapy, HIV infection has transformed from acute illness to a chronic, manageable disease. Age-related comorbidities in HIV-1 infected patients suggest accelerated aging. Astrocyte senescence has been shown to play a critical role in HIV-mediated premature aging. Our previous studies have demonstrated downregulation of SIRT1 expression in the brains of aged HIV transgenic (>15 months) rats compared with their age-matched controls. Current study was focused on the role of lncRNAs in cellular senescence with a focus on lncRNA TUG1, that has been shown to be upregulated in the human subventricular zone during aging. The role of lncRNA TUG1 in astrocyte senescence & the transcriptional regulation of SIRT1 however, remain unexplored. Our findings demonstrate that in HIV Tat stimulated A172 astrocytoma cells and human primary astrocytes there was upregulation of senescence markers (p21waf1 & p16INK4A) that is accompanied by cell-cycle arrest. We also observed increased senescence-associated β-galactosidase staining and increased expression of lncRNA TUG1 in the astrocytes, stimulated with HIV Tat compared with cells not exposed to the viral protein. Gene silencing of lncRNA TUG1 abrogated both HIV Tat-mediated downregulation of SIRT1 as well as concomitant upregulation of the senescent markers. Understanding the mechanism(s) underlying astrocyte senescence could be pivotal for future development of novel therapeutic strategies against HIV-associated premature aging involving glial senescence.

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T37 Mutations on human norepinephrine transporter at tyrosine 467 and human dopamine transporter at tyrosine 470 are critical for HIV-1 Tat-induced inhibition of dopamine transport

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The prefrontal cortex is an important brain region for higher cognitive function, where both norepinephrine (NE) transporter (NET) and dopamine (DA) transporter (DAT) play a critical role in reuptake of DA. Through computational 3D structure modeling, we predicted that tyrosine 467 on human NET (hNET), a unique binding site corresponding to tyrosine 470 on human DAT (hDAT), is critical for Tat binding. We have demonstrated that HIV-1 Tat directly inhibits DA uptake via DAT and that mutation of hDAT at tyrosine 470 attenuates Tat's inhibitory effect. The current study evaluated the mutational effects of the tyrosine 467 (Y467H, A, F) on basal DA and NE transport in the presence or absence of Tat. Compared to wild-type (WT) hNET, the Vmax of [3H]DA and [3H]NE uptake in Y467A was reduced by 70% and 86%, respectively, with no changes in the Km, whereas Y467H and Y467F retained a normal transport function for DA and NE. In addition, a 55% reduction of the Bmax of [3H]WIN35,428 binding in Y467A and in Y467H was observed with no changes in Kd. The Bmax of [3H]Nisoxetine in Y467A was decreased by 62% with no change in Kd, whereas both Y467H and Y467F did not alter the Bmax. Biotinylation studies showed that the decreased Vmax in Y467A is accompanied by a reduction of surface hNET. Importantly, Tat-induced decrease in DA uptake observed in WT hNET was attenuated in Y467H and Y467F. These results provide a novel mechanistic basis for identifying therapeutic targets on DAT and NET for developing compounds that specifically block Tat binding site(s) in the transporters.

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T38 Taar1 genotype associated with risk for methamphetamine intake correlates with dopaminergic changes

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Methamphetamine (MA) high drinking (MAHDR) and low drinking (MALDR) mouse lines were bred for amount of voluntary MA consumption. MAHDR mice have non-functional trace amine-associated receptor 1 (TAAR1), due to homozygosity for a single nucleotide polymorphism in the Taar1 gene, whereas MALDR mice possess functional TAAR1. MA is a TAAR1 agonist. Dopamine (DA) is a neurotransmitter acutely induced by MA and other drugs of abuse, and linked to reward. We tested whether the Taar1 mutation in MAHDR mice may dysregulate the DA system, by measuring the expression of a DA receptor, DRD1, and of the DA transporter, DAT. All mice received 2 mg/kg MA ip, and were euthanized at 15, 30, 60, 120 and 240 min. DRD1 and DAT were measured in the reward pathway regions, the pre-frontal cortex (PFC), ventral midbrain (VMB), and nucleus accumbens (NAc). In the NAc, MAHDR mice displayed an increase in DRD1, compared to MALDR mice. DAT levels in the NAc were decreased by MA in the MALDR, while increased in the MAHDR at 30 min. In PFC, DRD1 was decreased in both lines, but earlier in MALDR than in MAHDR mice. We also measured CCL5/RANTES, a chemokine transcriptionally regulated both by TAAR1 and by DA. CCL5/RANTES was decreased in the NAc of MALDR mice at 30 min, but in MAHDR mice, it was increased in VMB at 60 min. These results support other data suggesting that TAAR1 contributes to DA pathway activity, and suggest a role for the expression of CCL5/RANTES in MA intake, providing further understanding of molecular mechanisms of drug addiction and susceptibility to its consequences.

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T39* Protease Inhibitors Effects on Vascular Smooth Muscle Cells

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In the current era of combination antiretroviral therapy (cART), HIV-associated neurocognitive disorders (HAND) continue to affect the clinical outcome of HIV infection, even in the setting of systemic viral suppression. However, certain antiretroviral treatments (ART), particularly those including protease inhibitors (PI), may cause toxicity to brain vasculature via cerebral small vessel disease (CSVD) which in turn can lead to disturbance of cerebrovascular autoregulation manifested in subclinical cognitive deficits contributing to HAND. Several studies have been conducted on ART toxicities in vitro using neuronal, astroglial, and endothelial cultures. However, the effect of PI on vascular smooth muscle (VSMC), which play an important role in cerebral small vessels homeostasis, has not been fully characterized yet. In this study, we describe the effects of ART-PI combination of Ritonavir+ Lopanovir and Darunavir, on human VSMC. Gene expression and protein levels of different markers such as cellular aging p16, contractile alpha-smooth muscle actin (SMA) protein, Zmpste24, Lamin-A and inflammatory cytokines were analyzed after 24h, 48h and 72h exposure to antiretrovirals. Significant alterations of Zmpste24 were found in the presence of both ART-PI after 24 and 48 hours treatment. In addition, both PI antiretroviral drugs significantly decreased levels of SMA protein in VSMC, especially after 48 hours. These findings will help us to better understand the contribution PI in the context of HIV/ARV-PI comorbidity and develop potential neuroprotective therapeutic interventions

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T40 Modulating macrophage phenotypes as a therapeutic strategy in neurodegenerative diseases

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In the central nervous system (CNS), the balance of brain macrophage phenotypes play an important role in the inflammatory gene expression in neurodegenerative diseases such as HIV-associated neurocognitive disorders (HAND). Recent studies have shown that cannabinoids are associated with therapeutic effects in humans. In these studies, a cannabinoid receptor agonist, WIN55,212-2 (WIN) was tested for its ability to block IL-1β and Aβ-induced proinflammatory and neurotoxic phenotype and HIV replication efficiency in macrophages. In our preliminary studies monocyte-derived macrophages (MDM) were treated with WIN and exposed to the proinflammatory cytokine IL-1β and Aβ. MDM morphology was analyzed by fluorescent microscopy. To test neurotoxicity, primary human neurons were treated with MDM conditioned media from all treatment groups an analyzed for neuronal integrity. These HIV-relevant stimuli reduced expression of triggering receptor expressed on myeloid cells (TREM2), and increased IL-1β and IFN- . Interestingly, pretreatment of MDMs with WIN reversed these effects. WIN also altered the morphology of MDMs, promoting more elongated cells, indicative of M2 phenotype, and decreased p24 HIV-1 production. These results suggest that chronic HIV infection induces a proinflammatory and neurotoxic phenotype in brain macrophages. However, this may be reduced or blocked with cannabinoid receptor agonists.

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T41* MicroRNA cluster miR199a/214 are differentially expressed in female and male rats following nicotine self-administration

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Previous research has established sex differences associated with nicotine intake, however a significant gap in knowledge remains regarding the molecular mechanisms that govern these differences at the transcriptional level. One critical regulator of transcription are microRNAs that regulate an array of important biological functions altered in several disease states, including neuroadaptive changes associated with drug dependence. We examined the prefrontal cortex (PFC) from male and female Sprague-Dawley rats following self-administration (22 days) of nicotine or yoked saline controls using RNA-Seq technology and found an array of miRNAs to be significantly and differentially regulated by nicotine self-administration. Of these, we found the expression of miR-199a and 214, which are expressed on the same cluster of chromosome 1, to be upregulated in the female rats exposed to nicotine and further validated by RT-PCR. Bioinformatics analysis to assess common targets of miR-199/214 identified Sirtuin 1 (SIRT1), a nicotinamide adenine dinucleotide (NAD)- dependent deacetylase that plays a role in apoptosis, neuron survival, and stress resistance. Using western-blot, we confirmed downregulation of SIRT1 and increased cleaved caspase 3 expression in the brains of nicotine-exposed female rats and no change in expression levels in the other groups. Collectively, our findings highlight a miR-199/214 regulatory network , through SIRT1, may be associated with nicotine seeking in females which may serve as a potential therapeutic target for sex-specific treatment approaches.

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T42 Prescription opioids induce gut dysbiosis and exacerbate Inflammatory Bowel Disease

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Opioids are the most prescribed analgesics for pain in Inflammatory Bowel Diseases (IBD), however the consequences of opioid use on IBD severity is not well defined. We evaluated the histopathology of gut biopsy from opioid using and non-opioid using IBD patients. Significantly higher histological score with severe pathological damage were observed in opioid using when compared to non-opioid using IBD patients. To determine the detrimental effect of opioids on IBD severity we investigated the consequence of hydromorphone in a murine model of dextran-sodium-sulfate (DSS)-induced colitis. We show that hydromorphone and DSS independently induced epithelial barrier dysfunction, bacterial translocation, and increased inflammation, which were exacerbated in mice receiving hydromorphone in combination with DSS. Hydromorphone-treated DSS mice exhibited significant microbial dysbiosis with an expansion of Proteobacteria and Verrucomicrobia and reduced proportion of Firmicutes. Predictive metagenomic analysis revealed high abundance in the bacterial communities associated with virulence, antibiotic resistance, toxin production and inflammatory properties. We further observed that hydromorphone modulates tight junction proteins organization in a myosin light chain kinase (MLCK)-dependent manner. Treatment with ML-7, an selective MLCK inhibitor, ameliorates the detrimental effects of hydromorphone and decrease severity of IBD. These findings warrant a careful evaluation of the potential detrimental effects of prescription opioids on IBD severity and should be prescribed cautiously.

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T43 Molecular mechanisms of ZIKV induced alteration in developing brain

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Since its discovery around six decades ago, Zika Virus (ZIKV) has been considered a mild human pathogen, until recently when it was declared as major international public health concern following large outbreaks in various regions of the world and associated congenital disabilities. ZIKV possess high propensity towards neural stem cells (NSCs); it impairs proliferation and differentiation pattern of NSCs. Apart from infecting early NSCs, ZIKV also infects indispensable astrocytes in developing brain and induce an inflammatory response. We employed the most relevant model systems - human fetal neural stem cells (fNSCs), fNSC derived astrocytes and in-utero electroporated mouse brain. We observed that ZIKV Envelope (E) protein altered proliferation and differentiation of NSCs that was validated in animal model. We performed Next-generation sequencing for small RNAs to identify microRNAs modulated by E protein. At least 25 microRNAs were dysregulated and GO analysis indicated targets of these microRNAs are involved in cell cycle and development related processes. PAX3, NOTCH2 and WNT2 are targets of these differentially regulated miRNAs. On the other hand, E-protein induced activation of astrocytes and resulted in upregulation of GFAP and release of various inflammatory molecules-glutamate, ATP, and TNFα. In pursuit of the cellular and molecular mechanisms exploited by ZIKV, we found that ZIKV disrupts cell homeostasis by impairing properties of NSCs and triggering inflammation in developing brain by activating astrocytes.

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T44 Hippocampal cell loss in HIV-1 Tg rats: A Role for Impaired Neurogenesis?

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The Tat viral protein has been previously shown to decrease neuronal cell counts in the granular layer of the hippocampus; however, differences have not been shown in the HIV-1 Tg rat. We investigated the effects of S-equol treatment on hippocampal neurogenesis and morphology. Male and female HIV-1 Tg (n=42) or control (n=44) animals were orally given either 0.2 mg S-equol or a placebo from postnatal days 28 to 90. Brains were extracted and prepared for alternating sections at 300Î¼m of either Nissl or doublecortin (DCX) stain. DCX is expressed by immature neurons and is indicative of neurogenesis. Nissl stained hippocampal slices at approximately bregma 3.24mm were chosen to be analyzed under a light microscope to determine percent area of the dentate gyrus relative to the entire hippocampus, as well as the length and thickness of the dorsal and ventral blades of the granular layer. Total DCX neuron counts were found in the granular layer by taking 4 sequential hippocampal slices and using the optical fractionator probe to cover 100% of the region. An ANOVA statistical analysis revealed a significant 3-way interaction between Sex, genetic condition, and treatment group $p<.05$ for the percent area of the dentate gyrus. A sex difference was also found for the thickness of the granular layer $p<.05$. DCX neuronal counts resulted in impaired neurogenesis in the HIV-1 Tg rat compared to controls regardless of treatment group. S-equol failed to deter HIV neurogenesis impairments despite the increased synaptic connectivity previously reported.

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T45 Neuron-astrocyte interaction in pathogenesis of HIV-associated pain

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Pathological pain is a debilitating complication suffered by many HIV patients. However, the pathogenic mechanism remains unclear. Our previous studies on human postmortem tissues suggest the activation of astrocytes is specifically associated with the development of HIV-associated pain. Using the pain model generated by intrathecal injection of HIV-1 gp120, we have investigated the mechanism by which astrocytes are activated by gp120 in the mouse spinal cord. Our studies indicate that gp120-regulated Wnt5a from neurons plays a critical role in stimulating astrocytes, via its astrocytic co-receptor ROR2. We have further elucidated a ROR2-regulated MMP2/IL-1β pathway that feedbacks to regulate neuronal activity. Our findings reveal novel cellular and molecular mechanisms in the pathogenesis of HIV-associated pain.

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T46 HIV- Tat and Cocaine Impact Astrocytic DNA Methylation

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HIV infections and cocaine use are known to affect neuronal dysfunction. Astrocytes are the major regulators of energy metabolism in the central nervous system and mitochondria are the main producers of cellular energy by oxidative phosphorylation. Cocaine abuse and HIV infections are known to utilize brain energy reserves, and could possibly affect mitochondria in which DNA methylation, an important epigenetic modification has not been elucidated yet. We hypothesize that co-incubation of HIV-1 Tat with cocaine impact epigenetic modification of global DNA methylation, activating DNA methyl transferases (DNMT1, DNMT3a and DNMT3b) which affect the function of the regulator of mitochondrial biosynthesis, PGC-Î±1, and mitochondrial transcriptional factor (TFAM), thereby mediating disease progression. We addressed this in both in vitro as well as in vivo studies. Human astrocytes were exposed with either HIV-Tat Â± cocaine in vitro, and HIV-Tat (iTat) mice treated with Doxycycline (to induce Tat expression) and a combination of cocaine in vivo. The cell lysates and brain tissues respectively were isolated to study the epigenetic modification of DNA methylation and mitochondrial biogenesis. Global DNA methylation, DNMTs 1, 3a & 3b and mitobiogenesis of PGC-Î±1 and TFAM were analyzed. We observed significant decreases in global DNA methylation and DNMT expression in astrocytes treated with HIV-Tat + cocaine, as well as in brain tissues of HIV-Tat (iTat) mice treated with cocaine. These changes differentially regulated DNMT1 and DNMT3a, whereas PGC-Î±1 and TFAM were significantly upregulated, both effects accelerated by cocaine. Our results indicate that cocaine and HIV-Tat impacts astrocyte energy metabolism by altering DNMT expression, which might be a contributing factor towards the neurodegeneration observed in HIV-positive cocaine users.

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T47 Antiretroviral-mediated dysregulation of autophagy involves lysosomal impairment of microglia

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While combined antiretroviral therapy (cART) has significant impact on increasing the lifespan of HIV+ individuals, paradoxically, there is also an increased prevalence of HIV-associated neurocognitive disorders (HAND). Inflammation is a key underlying feature of HAND, with the role of antiretrovirals & also residual viral proteins implicated in this process. Microglia are extremely sensitive to a plethora of stimuli including viral proteins & cART. This study was aimed to investigate the molecular mechanism(s) underlying cART-mediated activation of microglia. Herein, we demonstrate that exposure of microglia to the three commonly used antiretrovirals - dolutegravir, tenofovir & emtricitabine (5Î¼M each) resulted in impaired lysosomal functioning involving increased lysosomal membrane permeabilization & decreased cathepsin D activity. Compromised lysosome functioning resulted in dysregulated autophagy, which was further confirmed by increased expression of LC3B & p62, leading in turn, to activation of microglia. Pretreatment of the cells to the lysosomal protecting agent - N-acetylcysteine (NAC) was found to reverse the damaging effects of cART. Also, cART-treated HIV transgenic rats demonstrated exaggerated lysosome impairment, dysregulated autophagy & increased microglial activation in the prefrontal cortices. Interestingly, pre-treatment of rats with NAC (200mg/Kg) mitigated deleterious effects of cART in vivo. Strategies aimed at lysosomal protection could thus be considered as adjunctive therapeutics for the treatment of HAND in HIV+ individuals on cART.

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T48* Suppression of Macrophage-Induced Neurotoxin Production via Estrogen Receptor Signaling.

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Macrophages and microglia (M/M) play pivotal roles in the pathogenesis of HIV-associated neurocognitive disorders. The ensuing inflammatory M/M activation causes neuronal damage. Studies utilizing exogenous therapeutics to mitigate disease progression have been unsuccessful; however, targeting endogenous pathways, such as estrogen signaling may be advantageous. 17b-estradiol, the most active form of estrogen, activates estrogen receptors and has been reported to inhibit HIV infection in primary macrophages and peripheral blood mononuclear cells and protect neurons against HIV proteins, gp120 and tat. The literature has also suggested that Secoisolariciresinol diglucoside (SDG), a flaxseed lignin, may interact with estrogen receptors. We have shown that SDG may suppress macrophage induced neurotoxicity and viral replication during neurotoxicity. Therefore, we hypothesize that 17b-estradiol and SDG may be neuroprotective during HIV infection and other inflammatory stimuli in an estrogen receptor dependent manner. To understand this, we stimulated or infected macrophages with HIVJAGO, respectively, in the presence and absence of increasing doses of 17b-estradiol or SDG. Whole cell and cytoplasmic lysates, mRNA and conditioned medium were collected at various time points. We found that 17b-estradiol and SDG suppressed neurotoxin production and viral replication from HIV infected macrophages in an estrogen receptor dependent manner. Given these studies, estrogen signaling may reduce oxidative stress and inflammation seen during HIV-associated neurocognitive disorders.

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T49 Metabolic Study of HIV CNS reservoirs

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The immunodeficiency virus (HIV) has become chronic disease due to the success of long-term anti-retroviral therapy (ART). However, at least half of the HIV infected population show a sign of cognitive impairment. Several groups, including ours, propose that damage is a product of bystander damage. Our goal here was to characterize the metabolomics of those viral reservoirs. We identified that T cells and macrophages with latent HIV integrated use mostly glutamate and glutamine as a significant source of energy to produce ATP. This is significant because glutamate is already dysregulated in the HIV CNS and is the most abundant neurotransmitter in the brain. Thus, viral reservoirs have an unlimited source of energy to survive for extended periods. Further characterization of the TCA indicates that several metabolites, before their processing in the mitochondria, are accumulated, including α-ketoglutarate. α-ketoglutarate is not only a TCA intermediary but also participates as a transcription factor. α-ketoglutarate alone can regulate HIV replication and silencing. In conclusion, we propose that these metabolic changes combined could help to kill viral reservoirs.

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T50 Wnt7a-MDMs regulate astrocyte phenotype and are associated with neuroprotection

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Monocyte-derived macrophages (MDM) display a myriad of phenotype and functions, and in the central nervous system contribute to neuroprotection or neurodegeneration. We described a unique MDM phenotype generated in response to Wnt7a; a member of the Wnt ligand family which orchestrate cell development, phenotype and function. We previously demonstrated that Wnt7a skews monocytes to differentiate into MDMs with a cytokine profile distinct from inflammatory and alternative MDM controls (M1 and M2a MDMs respectively), including elevated IL-6 secretion and reduced IL-10 and IL-12 secretion. Wnt7a-MDMs also display reduced CD11b expression, and reduced phagocytic capacity when compared to M1 and M2a MDM controls. We demonstrate here that conditioned media (CM) from Wnt7a-MDMs diminish astrocyte proinflammatory (A1) phenotype. Specifically, Wnt7a-MDMs reduced transcription of complement proteins C3 by five-fold and C4a and C4b by two folds relative to M2a-MDMs. Wnt7a-MDMs also reduced astrocyte expression of lipocalin-2 by approximately 300 folds relative to M1-MDMs. Wnt7a-MDMs secreted less C1q, a driver of A1 phenotype, than both MDM controls, and increased astrocyte proliferation relative to M1 MDMs. Lastly, in the context of HIV-Associated Neurocognitive Disorders (HAND), Wnt7a expression was consistently lower in the brain of HIV associated dementia (HAD) patients relative to seronegative and neurocognitively normal patients. Overall these data demonstrate that Wnt7a-MDMs reduces astrocyte inflammation and suggest that levels of Wnt7a in the CNS may be neuroprotective.

T51 Thalamic Substructures in HIV: Volume Deficits and Correlates

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The current standard of measuring global, undifferentiated brain structures is too coarse to distinguish substructural volumes, such as those of

the thalamus, which may be differentially affected by Human Immunodeficiency Virus (HIV). After informed consent, 52 healthy controls (~54yo, 25 women) and 17 adults infected with HIV (~59yo, 8 women) were scanned on a GE 3T MRI system to acquire WMn-MPRAGE images with 1mm isotropic spatial resolution. The multi-atlas used for thalamic parcellation comprised 20 WMn-MPRAGE datasets with manual delineations of thalamic nuclei performed by a neuroradiologist guided by the Morel atlas. Five thalamic structures, corrected for supratentorial brain volume, were considered: mediodorsal (MD), anterior ventral (AV), ventral lateral posterior (VLp), ventral posterior lateral (VPl), and pulvinar (Pul). Multiple regressions conducted for each substructure tested diagnosis, age, and their interaction. Age explained a significant portion of the variance in volumes of the AV (32%), VPl (20%), and Pul (33%) (all smaller with older age): MD was not influenced by either age or diagnosis. VLp was the only region affected by diagnosis, which explained 27% of the variance in its volume (smaller in HIV relative to controls). Within the HIV group only, smaller VLp volume was associated with lower CD4 prior nadir (rho=.65, p=.01). These results demonstrate the utility of using automatic segmentation of WMn-MPRAGE data to delineate the thalamic substructures and reveals the unique liability of VLp to HIV.

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T52 Long-term HIV-1 Tat expression in the brain led to neurobehavioral, pathological, and epigenetic changes reminiscent of accelerated ageing

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HIV infects the central nervous system and causes HIV/neuro AIDS, which is predominantly manifested in the form of mild cognitive and motor disorder in the era of combination antiretroviral therapy. HIV Tat protein is known to be a major pathogenic factor for HIV/neuro AIDS through a myriad of direct and indirect mechanisms. However, most, if not all of studies involve short-time exposure of recombinant Tat protein in vitro or short-term Tat expression in vivo. In this study, we took advantage of the doxycycline-inducible brain-specific HIV-1 Tat transgenic mouse model, fed the animals for 12 months, and assessed behavioral, pathological, and epigenetic changes in these mice. Long-term Tat expression led to poorer short-and long-term memory, lower locomotor activity and impaired coordination and balance ability, increased astrocyte activation and compromised neuronal integrity, and decreased global genomic DNA methylation. There were sex- and brain region-dependent differences in behaviors, pathologies, and epigenetic changes resulting from long-term Tat expression. All these changes are reminiscent of accelerated ageing, raising the possibility that HIV Tat contributes, at least in part, to HIV infection-associated accelerated ageing in HIV-infected individuals. These findings also suggest another utility of this model for HIV infection associated accelerated ageing studies.

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