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PLENARY LECTURERS

Drugs, Brains and Cars: Bench to Roadside

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Licit drugs intended to treat disease may impair driver performance at therapeutic levels, increasing the risk of driver safety errors that lead to vehicle crashes. We need reliable data to advise patients on whether or not to drive, yet dose-response effects of different drugs on driving are often unknown. A general framework for evaluating these effects relies on a functional evaluation of multiple domains (cognitive, motor, perceptual, psychiatric) that are important for safe driving and can be applied across many disorders and medications, that provides indirect evidence to evaluate driver safety. Driving simulation and road tests (state sanctioned, instrumented vehicles, naturalistic studies) provide more direct and specific evidence to evaluate driver safety. These complementary sources of evidence play distinct roles in evaluating immediate, mid-term and long-term effects of drugs on driving safety.

Standard deviation of lane position (SDLP), measured in simple scenarios in driving simulator or a real car, has been promoted as a key index of drug safety in clinical trials. However, SDLP depends primarily on vigilance and vasomotor control and does not tap into key abilities (decision making, awareness, emotion, working memory) that are essential for safe driving. The relationship of SDLP to real world driver safety outcomes (e.g., state crash records, traffic citations, "gold standard" state sanctioned road test) is unclear. Efforts to link driver metrics and blood alcohol concentration (BAC) to inform drug evaluations are subject to accuracy of alcohol based crash statistics (calculated per 1 million or 100 million miles traveled), high variability of crash risk around legal levels of intoxication, and biological differences between alcohol and most drugs being interrogated.

Clinical trials of drug effects on driver safety require clear and objective summary outcome measures, and no single performance factor can possibly capture the diversity of drug effects on driving. An evidence-based, pragmatic set of easily obtained performance measures is needed to address this pressing real-world issue.

Gabapentin as a Novel Treatment for Alcohol Dependence

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Alcohol use disorders can contribute to the spread of HIV/AIDS, negatively impact treatment response in infected individuals and contribute to liver disease and other disorders that can impact the progression of HIV infections. FDA-approved medications for alcohol dependence are prescribed for less than 9% of US alcoholics. We sought to determine if gabapentin, a widely-prescribed generic calcium channel/ γ -aminobutyric acid-modulating medication that is not metabolized in the liver, increases rates of sustained abstinence and no heavy drinking and decreases alcohol-related insomnia, dysphoria and craving, in a dose-dependent manner.

A 12-week, double-blind, placebo-controlled, randomized dose-ranging trial was conducted in 150 men and women older than 18 years with current alcohol dependence. Oral gabapentin (0, 900 or 1800 mg/d) and weekly, individual concomitant manual-guided counseling were provided for all participants. Main outcome measures were rates of complete abstinence and no heavy drinking (coprimary) and changes in mood, sleep and craving (secondary) over the 12-week study.

Gabapentin significantly improved the rates of abstinence and no heavy drinking. The abstinence rate was 4.1% in the placebo group, 11.1% in the 900-mg group, and 17.0% in the 1800-mg group ($P = 0.04$ for linear dose effect, number needed to treat [NNT] = 8 for 1800 mg). The no heavy drinking rate was 22.5% in the placebo group, 29.6% in the 900-mg group, and 44.7% in the 1800-mg group ($P = 0.02$ for linear dose effect: NNT = 5 for 1800 mg). Similar linear dose effects were obtained with measures of mood ($P = 0.001$), sleep ($P < 0.001$), and craving ($P = 0.03$). There were no serious drug-related adverse events, and terminations owing to adverse-events (9 of 150 participants), time on study (mean 9.1 weeks) and rate of study completion (85 of 150 participants) did not differ among groups.

In summary, gabapentin (particularly the 1800-mg dosage) was effective in treating alcohol dependence and relapse-related symptoms of insomnia, dysphoria and craving, with a favorable safety profile. That gabapentin is not appreciably metabolized in the liver may be an advantage for patients with liver dysfunction, and it is not known to interfere with the metabolism of commonly used illicit or prescribed drugs. Increased implementation of pharmacological treatment of alcohol dependence may be a major benefit of gabapentin as a treatment option for alcohol dependence. Supported by NIAAA, grant number R37AA014028. TRIAL REGISTRATION: [clinicaltrials.gov Identifier: NCT00391716](https://clinicaltrials.gov/Identifier/NCT00391716).

Chemokine receptor signaling regulates neural stem cell function in the developing and adult nervous system.

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It is now known that neural stem cell niches exist in the adult brain and contribute to ongoing neurogenesis throughout life. Neurogenic activity has been described in both the subventricular zone (SVZ) and the subgranular zone of the dentate gyrus (SGZ). Chemokine signaling through the CXCR4 and CXCR7 receptors has been shown to direct the migration and development of neural stem cells during embryogenesis and in the context of adult brain repair. CXCR4 signaling can influence the development of radial glia in the embryonic brain and the chemokine SDF-1/CXCL12 can be transported by radial glia to different parts of the developing nervous system. In the adult brain CXCR4 signaling can cooperate with the neurotransmitter GABA to direct the development of neural stem cells. For example, following ischemic stroke neural stem cells migrate from the subventricular zone to the stroke penumbra where they are integrated into neuronal circuits. Early synapses established by these young neurons use excitatory GABAergic signaling that is enhanced by the simultaneous action of SDF-1.

We have now defined another neurogenic region in the adult brain which we have named the subhippocampal zone (SHZ). Using a CXCR4-EGFP reporter mouse we observed the presence of a pool of EGFP expressing cells in a previously unrecognized neurogenic zone in the medial wall of the lateral ventricle covering the dorsal surface of the hippocampus. These cells first exit the lateral ventricles at the fimbria-dentate junction; navigate the hippocampal fimbria as a stream of migratory cells—ultimately reaching the meninges. The cells then closely migrate along the meninges in the direction of the DG. Deletion of CXCR4 from neural stem cells (NSCs) or neuroinflammation induced by HIV-1 resulted in the appearance of neurons in the DG which were the result of migration of NSCs from the SHZ. Some of these neurons were ectopically placed. Our observations indicate that the SHZ is a neurogenic zone in the adult brain through migration of NSCs. Regulation of CXCR4 signaling in these cells may be involved in repair of the DG and may also give rise to DG ectopic neurons in the context of neuropathology.

Speaker Abstracts By Symposium

SYMPOSIUM #1: Exosomes and Neurolmmune Interactions

The exosome-secretory pathway transports amyloid precursor protein carboxyl terminal fragments from the cell into the brain extracellular space

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In vitro studies have shown that cultured neurons secrete exosomes containing full-length amyloid precursor protein (fAPP) and APP processing products, carboxyl-terminal fragments (APP-CTFs) and amyloid β , an amyloidogenic peptide implicated in the pathogenesis of Alzheimer's disease. We investigated the secretion of fAPP and APP-CTFs via the exosome secretory pathway *in vivo*. We developed a novel protocol to isolate exosomes from the extracellular space of murine and human brain tissue, demonstrating that brain tissues frozen and kept at -80°C for long periods of time yield exosomes with the same characteristics as exosomes isolated from freshly removed mouse brains. In accordance with the high levels of fAPP and APP-CTFs in the brain of transgenic mice overexpressing human APP (Tg2576), exosomes secreted into the brain extracellular space of these mice contain higher levels of fAPP and APP-CTFs than exosomes secreted in the brain of wild-type littermate mice. The ratio of APP-CTFs to fAPP is higher in brain exosomes compared to brain homogenates in both Tg2576 and non-transgenic mice. These data show that the amount of fAPP and APP-CTFs secreted from the cell by brain exosomes is prorated to their brain levels and brain exosomes are specifically enriched with APP-CTFs regardless of levels of APP expression. Thus, the exosome-secretory pathway plays a pleiotropic role in the brain; exosome secretion is beneficial to the cell acting as a specific releasing system of the neurotoxic APP-CTFs and amyloid β but the secretion of exosomes enriched with APP-CTFs, neurotoxic proteins that are also a source of secreted amyloid β , is harmful to the brain. Supported by the National Institutes of Health (AG017617) and the Alzheimer's Association (ZEN-10-172559).

Functional Exosomes from HIV-1 and HTLV-1 infected cells: tails of survival and death

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Recently, much interest has developed regarding mechanisms of extracellular delivery of nucleic acids and proteins among virally infected and recipient cells. While the role of exosomes in viral pathogenesis and disease states remains largely unknown, it is now widely accepted that exosomes play important roles in intercellular communication, cellular inflammation, antigen presentation, programmed cell death, and pathogenesis. We have previously reported that HIV-1 encodes its own miRNAs that regulate viral and host gene expression. The most abundant HIV-1-derived miRNA, also reported by others using deep sequencing, is the TAR (Trans-Activation Response element) miRNA. We have recently found the presence of TAR RNA in exosomes from cell culture supernatants of HIV-1 infected cells and patient sera. We report that prior exposure of naïve cells to exosomes from infected cells increased susceptibility of the recipient cells to HIV-1 infection. Exosomal TAR RNA down regulated apoptosis by lowering Bim and Cdk9 proteins in recipient cells with copy numbers of 10^4 - 10^6 copies/ml of TAR. TAR RNA in the serum exosomes of highly active antiretroviral therapy (HAART)-treated patients or long term nonprogressors (LTNPs) also showed 10^3 copies/ml. Very recently, we have found that TAR is able to activate cytokines in the recipient cells by increasing the nuclear accumulation of both p65 and p50 (component of NFkB complex). This increase may be related to a newly formed IKKb in the TAR treated cells which may be the result of TLR3, 7 and 8 activation by TAR. Interestingly the TAR effect on TLR3 is functional in NFkB reporter assays.

Human T-lymphotropic virus type 1 (HTLV-1) is the causative agent of adult T-cell leukemia (ATL) and HTLV-1-associated myelopathy (HAM)/tropical spastic paraparesis (TSP). The HTLV-1 transactivator protein Tax controls many critical cellular pathways including host cell DNA damage response mechanisms, cell cycle progression, and apoptosis. We recently found that exosomes derived from infected cells contained the oncogenic Tax protein and pro-inflammatory mediator IL-6, as well as the viral mRNA transcripts, tax, hbx, and env. We also observed drastic reduction of MCP-1 and RANTES in exosomes from infected cells. Furthermore, we observed that exosomes released from HTLV-1 infected Tax-expressing cells contributed to enhanced survival of target cells when treated with FAS antibody. IL-2 dependent CTLL-2 cells that received Tax containing exosomes were also protected from apoptosis. Similar experiments with PBMCs also showed protection of the cells in 15 day cultures in the absence of PHA/IL-2. Collectively, these data suggest that exosomes from retrovirally infected cells control the recipient bystander cells in creating a “proinflammatory environment” similar to what is observed in the pathogenesis related to infection.

Activated monocyte-derived exosomes alter neural cell function

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We recently reported that 60% of subjects with HCV and virally-suppressed HIV infection (coinfection) were cognitively impaired. Importantly, we showed that peripheral monocytes in coinfection had a distinct type-I interferon (IFN) activation profile similar to treated HIV monoinfection. The mechanism for a subtle change in cognition may involve dysregulation in cell-cell communication rather than frank brain activation with subsequent neural cell death. Exosomes are membrane-bound vesicles shed by cells that can transfer host RNA, miRNAs and proteins to recipient cells. We hypothesized that exosomes from coinfecting subject monocytes could transfer miRNAs (miR) to neural cells and subsequently disrupt their function. We profiled monocyte-derived exosomes from HIV/HCV-coinfecting subjects using miR microarrays and found the vesicles highly enriched in miR-223. Further, we developed an *in vitro* model of this activation by treating monocytes with IFN α . The miR content of exosomes shed by IFN-activated monocytes recapitulated the findings from coinfecting subjects in that miR-223 was the most abundant exosomal miR. These exosomes were readily engulfed by both primary neurons and astrocytes. We determined, using astrocytoma cells that IFN α -treated monocyte-derived exosomes transferred functionally active miR-223. Using gene expression arrays and IPA analysis, we determined that several neural cell-related genes were suppressed in astrocytes overexpressing miR-223. One target, CY26B1, a cytochrome p450 enzyme, critical to retinoic acid metabolism in the brain was suppressed in exosome-treated astrocytes as well. We conclude that monocyte-derived exosomes from both HIV/HCV coinfecting individuals and IFN-treated monocytes can be internalized by neural cells and functionally alter their homeostasis. This new mechanism may explain subtle cognitive impairment observed in coinfection.

Exosome-mediated shuttling microRNAs: effects on neurons

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Exosome-mediated shuttling microRNAs: effects on neurons Extracellular vesicles such as exosomes have recently emerged as important mediators of cell-to-cell communication in the brain. Exosomes carry a mixed cargo of lipids, proteins and nucleic acids and when released into the target cell, are able to trigger downstream molecular pathways. We are particularly interested in understanding the effect of such exosome-carried microRNAs (miRNAs) on neurons. In particular, we are interested to learn about miRNAs released by macrophages in the presence of methamphetamine (METH). We found that treatment of mouse hippocampal neurons with certain miRNAs carried in exosome mimics can trigger cell death Intriguingly, these miRNAs are significantly increased in exosomes released from primary human macrophages that have been exposed to methamphetamine (METH). We believe that these miRNAs are potential neurotoxic factors and are specifically released during METH-induced insult on the brain. In summary, we identify a novel route by which METH can trigger neurotoxic pathways in the brain.

Role of exosomes from HIV-1 infected cells in neurodegeneration

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Neuronal dysfunction and degeneration are the causative mechanisms for the HIV-associated neurocognitive disorders (HAND) in the era of combined antiretroviral therapy (cART). In this study we assessed the effects of exosomes derived from PMA activated promonocytic cells U1 that are latently infected with HIV-1 and parental cells U937 on human fetal neurons. Neuronal cultures treated with U1 cell derived exosomes were found to be severely compromised in their ability to maintain existing neuronal network as well as their ability to form neurites in a scratch-wound assay. In addition, neuronal cultures treated with U1 exosomes demonstrated increase in reactive oxygen species (ROS) generation and low levels of superoxide dismutase (SOD) activity indicating heightened oxidative stress. Analysis of the phosphorylation status of protein kinases in neurons treated with U1 exosomes using phosphoproteome array demonstrated dysregulation of regulators of signal transduction, cell proliferation and survival. Specifically, we observed dysregulation of cAMP response element binding protein (CREB)-target gene expression. Collectively, these observations demonstrate that exosomes derived from HIV-1 infected cell can cause neuronal dysfunction and degeneration.

Microvesicles released from astrocytes regulate the peripheral immune response to CNS inflammation

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The mechanism by which brain inflammation drives the systemic immune response is currently unknown. In this study we provide evidence that this neuroimmune axis involves microvesicles released from brain that enter into peripheral circulation. IL-1 β , TNF α and ATP evoked a rapid and robust efflux of microvesicles from cultured astrocytes by mechanisms that involved a stabilization of nSmase2 into ceramide-rich membrane microdomains. Microvesicle release was blocked by inhibition of IL-1 β receptor binding, or inhibition of ceramide formation. These microvesicles contained a complex lipid and protein content that was consistent with exosomes. Using an *in vitro* blood brain barrier (BBB)-model prepared with BmuEC with GFAP-GFP expressing astrocytes we determined that microvesicles carrying GFP as cargo readily crossed endothelial cells with intact tight junctions. Electron microscopy demonstrated that the majority of exosomes appeared to readily cross endothelial membranes as intact particles and were released into the circulation. These particles distribute widely in the periphery and regulated leukocyte trafficking into the CNS through mechanisms dependent on peroxisome proliferator-activated receptor gamma. These data suggest that lipoprotein complexes (exosomes) released from astrocytes readily cross the BBB, and enter into circulation where they regulate the peripheral leukocyte response to CNS inflammation.

SYMPOSIUM #2: Pharmacology and Signaling in Drug Abuse

New concepts in opioid receptor signaling

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Opioid receptors comprise mu, delta and kappa types and are selectively activated by endogenous opioid peptides. The mu-receptor is responsible for the majority of clinical effects of opiate drugs, including pain relief and addiction liability. Opioid receptors are 7-transmembrane G protein coupled receptors (GPCRs) and interact with heterotrimeric G proteins of the Gi/o family. Activation of opioid receptors triggers a series of downstream signaling events including inhibition of adenylate cyclase, stimulation of the mitogen-activated protein kinase (MAPK) pathway, opening of inwardly rectifying potassium channels and closing of calcium channels. Following activation the receptors are phosphorylated by kinases, usually by G protein coupled receptor kinases (GRKs) but also by other kinases, (e.g. protein kinase C), followed by beta-arrestin recruitment, internalization and recycling. Beta-arrestin also functions as a signaling molecule. However, the various signaling pathways and their relative importance downstream of opioid receptors can be modulated in a cell- and agonist-specific manner, potentially altering physiological outcomes. Modulation at the level of the receptor can arise from heterodimerization between opioid receptors or between opioid receptors and other GPCRs, including chemokine receptors, by agonist dependent signaling (signaling bias) or by the action of ligands at allosteric sites. Similarly, signaling can be controlled by the action of accessory proteins downstream of the receptor, such as Regulator of G protein Signaling (RGS) proteins. Supported by NIH.

Forgiving the sins of the father: Epigenetic inheritance of a cocaine resistance phenotype

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A rat model was developed in order to delineate a heritable phenotype resulting from the self-administration of cocaine. Delayed acquisition and reduced maintenance of cocaine self-administration was observed in male, but not female, offspring of sires that self-administered cocaine. Brain-derived neurotrophic factor (BDNF) mRNA and protein were increased in the medial prefrontal cortex (mPFC) and there was an increased association of acetylated histone H3 with BDNF promoters only in the male offspring of cocaine-experienced sires. Administration of a BDNF receptor antagonist (the TrkB receptor antagonist ANA-12) reversed the diminished cocaine self-administration in male cocaine-sired rats. In addition, the association of acetylated histone H3 with BDNF promoters was increased in the sperm of sires that self-administered cocaine. Collectively, these findings indicate that voluntary paternal ingestion of cocaine results in epigenetic reprogramming of the germline resulting in profound effects on mPFC gene expression and resistance to cocaine reinforcement in male offspring.

Nicotine Enhances Excitability of Medial Habenular Neurons via Facilitation of Neurokinin Signaling

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The medial habenula densely expresses nicotinic acetylcholine receptors (nAChRs) and influences nicotine-related behaviors, including nicotine intake and nicotine withdrawal. Although specific nAChR subunits are known to be involved in withdrawal behavior, the exact cellular mechanisms through which nicotine acts to cause this aversive experience is unclear. We demonstrate an interaction between the nicotinic and neurokinin signaling systems that may form the basis for some of the symptoms experienced during nicotine withdrawal. Using patch clamp electrophysiology in mouse brain slices, we show that nicotine increases the intrinsic excitability of medial habenular neurons. This nicotine-induced phenomenon requires activation of $\alpha 5$ -containing nAChRs and depends on intact neurokinin signaling. Microinjection of neurokinin 1 and neurokinin 3 receptor antagonists into the medial habenula is sufficient to induce withdrawal behavior in chronic nicotine-treated mice. Further, chronic nicotine reduces nicotine's ability to acutely modulate intrinsic excitability. Our work elucidates the interplay between two neuromodulatory signaling systems in the brain through which nicotine acts to influence intrinsic excitability. More importantly, we document a neuroadaptation of this mechanism to chronic nicotine exposure, and collectively implicate these mechanisms in the emergence of nicotine withdrawal behavior. Supported by DA171732, DA029157 & U19 - CA134682.

Targeting the endocannabinoid hydrolyzing enzyme ABHD6 to treat seizures and control neuroinflammation

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In the brain, a major role of the endocannabinoids (eCBs) signaling system is to control presynaptic neurotransmitter release and neuroinflammatory responses. This signaling system comprises the CB1 and CB2 receptors, and two main eCB, anandamide and 2-AG, which are produced and inactivated by distinct lipases and hydrolases. We recently demonstrated that the serine hydrolase ABHD6 is a bona fide member of the eCB signaling system that controls the availability of 2-AG. Specifically in neurons, ABHD6 is located post-synaptically, at the site of 2-AG synthesis, where it fine-tunes the stimulated production of 2-AG and the resulting activation of presynaptic CB1 cannabinoid receptors. Epilepsy is a common condition that is refractory to current therapies in approximately 30% of patients and is associated with pathologic cortical excitability and allied neuroinflammation. However, the therapeutic benefit of current treatments is limited by a lack of efficacy and their side effect profiles, underlining the need for developing new pharmacological strategies for the treatment of this disease. Targeting neuromodulatory signaling systems, like eCB signaling may provide greater efficacy and tolerability for the treatment of epilepsy. We found that ABHD6 inhibition decreases pentylenetetrazole (PTZ)-induced seizure incidence, severity and mortality, as well as spontaneous seizures occurring in R6/2 mice, a genetic model of juvenile Huntington's disease (HD) featuring dysregulation of eCB signaling. The involvement of the hippocampus in the seizures exhibited by R6/2 mice is suggested by interictal epileptic discharges and a neuroinflammatory response typified by increased Iba-1 and S100A immunostaining. We conclude that ABHD6 inhibition represents a novel antiepileptic strategy and approach to control neuroinflammatory response. Supported by NIH (DA026430) and CHDI.

Intracellular mechanism of methamphetamine regulation of dopamine transporter

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Methamphetamine addiction is a major public health problem with no effective treatment strategies or FDA-approved pharmacotherapies. Psychostimulant addiction, in general, is a chronic disease rooted in numerous drug-induced neurobiological adaptations. Arguably, the greatest promise in treatment of psychostimulant abuse lies in determining the underlying molecular mechanisms of these adaptations and developing targeted therapies. Methamphetamine is both a substrate for dopamine transporter and a high affinity ligand for an intracellular chaperon protein, sigma-1 receptor. Therefore, methamphetamine may exert its effects on the transporter both directly at the membrane and indirectly via activation/inhibition of intracellular targets. Upon ligand stimulation, the sigma-1 receptor translocates from its primary endoplasmic reticulum localization to other subcellular compartments and plasma membrane, where it regulates a variety of functional proteins including membrane proteins. Initially, methamphetamine interacts with the dopamine transporter directly by inducing an inward-facing conformation of the transporter, leading to blockade of dopamine uptake and increasing dopamine efflux. Following its entry into the dopamine neuron through the dopamine transporter, methamphetamine indirectly influences the activity of the transporter via initiation of an interaction between sigma-1 receptor and the dopamine transporter. This talk focuses on intracellular mechanism of methamphetamine regulation of dopamine transporter. Supported by NIDA/R01DA026947

Symposium #3: Inflammation: At the Core of Substance Abuse-Disease Spectrum Interaction

Alcohol Interaction with SIV Disease Progression, Translating Our Basic Science Findings to the Clinical Setting

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Alcohol abuse is the most common and costly form of drug abuse in the United States. The contribution of alcohol use disorders (AUD) to risky behaviors associated with greater incidence of human immunodeficiency virus (HIV) infection has long been recognized. With HIV becoming a more chronic disease since the advent of antiretroviral therapy, it is expected that AUD will have an adverse effect on the health of HIV-infected patients. The biomedical consequences of AUD are multi-systemic. Comorbid and pathophysiological conditions resulting from HIV infection in individuals with AUD indicate altered susceptibility to infection, host defense, and tissue injury, with a significant impact on disease progression. Organs particularly affected by the combined insult of alcohol abuse and HIV infection include the CNS, the immune system, the liver, heart lung, and the musculoskeletal system. This presentation will provide an overview of the principal pathological consequences of alcohol abuse in the HIV-infected individual, with particular emphasis on its impact on immunomodulation, erosion of lean body mass associated with AIDS wasting, and lipodystrophy. The implications for gender and age in the comorbid pathological effects of chronic alcohol abuse on disease progression will be discussed. In addition, it will discuss translational

initiatives to adapt evidence based behavioral interventions to reduce or avoid alcohol abuse in HIV patients with the goal of decreasing morbidity and improving outcomes in people living with HIV/AIDS. Supported by NIAAA-07577, NIAAA-09803, UAA021995A, and AA-11290.

Cannabinoids and HIV Disease: Neuroimmune substrates and sex differences in non-human primates and rodents

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Cannabinoid receptors are expressed on cells of the immune system, and recent research from our laboratory and others show that cannabinoids can impact HIV disease progression. However, there are also clear indications that this class of substances produces sexually dimorphic effects, including those that relate to HIV disease progression. For example, in male rhesus macaques inoculated with simian immunodeficiency virus (SIVmac251, 100 TCID₅₀/ml, i.v.), we found that chronic delta-9-tetrahydrocannabinol administration (0.32 mg/kg, i.m., twice daily, THC/SIV+) ameliorated disease progression, attenuated viral load and tissue inflammation, promoted retention of body mass, and reduced morbidity and mortality compared to male rhesus administered vehicle (VEH/SIV+). In contrast, an identical protocol of chronic THC in female rhesus macaques inoculated with SIV produced higher plasma viral loads, lower cell counts of both CD4+ and CD8+ T lymphocytes, net weight loss, and a shorter average time to death (9.6 months median survival) compared to the VEH/SIV+ group. These data are also concordant with rodent data from the laboratory indicating that the female sex hormone estradiol can attenuate the expression of a co-chaperone responsible for the level of CB-1 receptors at the cell membrane (i.e., AHA-1), as well as the effects of THC on complex behavior. Taken together, these data indicate that estrogen signaling in the brain opposes the effects of the cannabinoids and is likely responsible for reducing their protective effects on HIV disease progression in females. Supported by National Institute on Drug Abuse/DA019625 (PJW), DA020419 (PEM), DA031596 (CMF).

Traumatic Brain Injury Increases Alcohol Drinking and Promotes Neuroinflammation in Rats

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Traumatic brain injury (TBI) costs the U.S. \$16.5 billion and promotes alcohol abuse, but the underlying mechanisms for this effect are not known. This experiment aimed to [1] characterize post-TBI alcohol drinking and anxiety-like behavior in rats, and [2] assess neuroinflammation in the cortex of TBI rats. Our working hypothesis is that TBI promotes neuroinflammatory events that mediate escalated alcohol drinking and anxiety-like behavior. Male Wistar rats were trained to self-administer alcohol in 30-min daily operant sessions over 4 wks. Once responding stabilized, rats experienced either Craniotomy + TBI (n=19), Craniotomy alone (n=20), or no manipulation (Naive; n=12), and were tested for alcohol responding and anxiety-like behavior over 3 wks post-TBI. Animals were sacrificed and neuroinflammatory markers CD68 (microglia activation) and GFAP (astrocyte activation) measured via immunohistochemistry (IHC). TBI severity and baseline alcohol drinking each positively correlated with post-TBI increases in alcohol drinking, with the largest post-TBI increases in alcohol drinking occurring in moderate TBI rats with high baseline drinking. Moderate TBI animals also exhibited higher anxiety-like behavior than control groups. TBI rats exhibited diffuse astrocyte and microglia activation near site of injury. In our study, rats with the highest pre-injury alcohol drinking levels exhibited the largest post-TBI escalation of alcohol drinking, identical to human reports. These results show we have a model suitable for exploring mechanisms of post-TBI escalation of alcohol drinking. This work was supported by NIAAA grants 007577 and 018400.

Alcohol and an Oncogenic Herpesvirus - Potential Partners in Cancer Pathogenesis

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KSHV (Kaposi sarcoma-associated herpesvirus) is the most common etiologic agent of cancer for HIV-infected patients worldwide, and systemic KSHV replication is associated with tumor formation and progression. There are currently no viable approaches for reducing KSHV replication and KS risk, and identification of modifiable factors

contributing to KSHV pathogenesis is crucial for tumor prevention efforts. Oxidative stress modulates KSHV pathogenesis in cell culture and animal model systems, and alcohol induces systemic and regional oxidative stress. Therefore, we initiated a pilot study to identify relationships between KSHV infection, oxidative stress, and alcohol within a minority-predominant HIV+ cohort in New Orleans exhibiting high prevalence for both alcohol use disorders and KSHV infection. We also sought to determine whether alcohol modifies oxidative stress in cell culture systems relevant to KS. Thus far, plasma signatures indicate significantly increased systemic oxidative stress within HIV+ patients in our cohort relative to HIV-negative patients independent of either HIV replication or immune status and despite the prevailing use of antiretroviral therapy. In vitro studies also suggest that alcohol modifies redox status for primary endothelial cells - the cell of origin for KS tumors. These and additional data rationalize ongoing work to explore mechanistic and clinical relationships between alcohol and KSHV replication, with the hope of developing novel strategies for reducing KSHV-associated cancer risk in patients with alcohol use disorders. Supported by NIH/R01-CA142362/UL1-TR000165

Reduction of Excessive Drinking in Alcohol-Dependent Rats via Soluble Epoxide Hydrolase Inhibition

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Alcoholism (or alcohol dependence) is a chronic relapsing disorder characterized by compulsive drinking and a dysregulation of affective states that may promote an acceleration of drinking in the context of negative reinforcement. The development of neuropathic pain in human alcoholics may engender such emotional distress and promote drinking. Hyperalgesia is also observed in animal models of dependence as nociceptive thresholds are lowered following excessive (but not limited) alcohol exposure, although the exact mechanisms linking pain and the motivation to drink are unknown. Epoxyeicosatrienoic acids (EETs) are lipid mediators that exhibit analgesic and anti-inflammatory properties. As EETs are inactivated via soluble epoxide hydrolase (sEH), inhibition of sEH is considered a valid therapeutic strategy targeting a variety of inflammatory disease states affecting numerous physiological systems. Interestingly, sEH is expressed throughout the brain, primarily in astrocytes, although the enzyme is also enriched in neurons of the central amygdala, a region hypothesized to mediate both the affective dimension of pain as well as alcohol dependence. In the current study, we found that systemic sEH inhibition (sEH 1728, 0.3-1.0 mg/kg) reduced excessive alcohol self-administration in animals made dependent via chronic intermittent alcohol vapor exposure. Importantly, sEH inhibition did not affect drinking in non-dependent animals or water intake. These data suggest that systemic elevation of EETs may prove beneficial in reducing the compulsive drinking observed in alcoholics. Supported by AA020839 (SE) AR062866 (BI, BDH) AA008459 (GFK)

Symposium #4: Drug Abuse, Neuroimmunology and HIV/AIDS: Perspectives from Addiction Neuropharmacologists

Persistent inflammatory effects of methamphetamine on the blood-brain barrier and neurotoxicity

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Past and present studies have shown that oxidative stress, metabolic compromise, and excitotoxicity are contributory mechanisms that converge to mediate the neurotoxicity of methamphetamine to dopamine and serotonin terminals. The results from these studies will be briefly summarized. More recent experiments indicate that inflammatory mechanisms also contribute to the toxic effects of methamphetamine and that the co-morbidity of chronic environmental stress and methamphetamine synergize to produce long-term decreases in dopamine and serotonin content in the brain, an increase in blood-brain barrier permeability, and decreased immunoreactivity of endothelial tight junction proteins, all of which are blocked by the anti-inflammatory drug and cyclooxygenase inhibitor, ketoprofen. Moreover, recent findings will be presented that indicate peripheral organ injury and ammonia also contribute to the toxic effects of methamphetamine. The implications of peripheral organ injury and its contribution to the neurotoxic effects of methamphetamine will be discussed. Supported by NIH-DA07606; DA035499

Chronic psychostimulant exposure and HIV-1 Tat-induced neuronal dysfunction

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Drug addiction is a chronic condition that results in complex, interactive, and enduring maladaptations manifested throughout the individual, and it reflects an array of reward-mediated neurocognitive processes. For example, impulsivity and risk-taking are often initial motivators for abusing drugs; these traits are also associated with the tendency to engage in unprotected sexual activity. Accordingly, drug abuse is a risk factor for becoming infected with HIV. The comorbid condition presents an augmented neuropathological profile as well as increased morbidity and mortality over that seen in either drug abuse or HIV infection alone. Our laboratory is interested in determining how psychostimulant abuse alters the neuropathogenic profile of HIV-1 neurotoxic proteins. Our focus is on brain regions that regulate reward-mediated behaviors and are known to be dysregulated in both drug abuse and in neuroAIDS, e.g., the medial prefrontal cortex. To capture aspects of the chronic and system-wide maladaptations that occur in drug-abusing humans, we use chronic *in vivo* treatment protocols in laboratory rats. In one set of experiments, we evaluated consequences of cocaine self-administration on responding of cortical neurons to the HIV-1 toxic protein, Tat, and whether neurophysiological changes in cocaine-withdrawn rats were associated with behavioral indices of craving. We revealed that the ability of Tat to activate cortical neurons was greatly enhanced in rats with a cocaine history; indeed, the enhancement was excessive to the extent of inducing a pathological, over-activated state. Aspects of the Tat-induced pathophysiology were correlated with cocaine-craving, suggesting that motivational drives that promote drug-taking may be enhanced by the HIV-1 protein. Thus, it may be more difficult for the comorbid individual to abstain from cocaine. In another set of experiments, we determined that high voltage activated L-type calcium channels, which are key players in cortical neuron excitability, can be excessively upregulated by both Tat and chronic cocaine exposure. Pharmacological antagonism of these channels was sufficient to restore normal function. These findings point to a potential pharmacotherapy that may be beneficial in reducing craving in the cocaine-abusing HIV-infected individual. This work was supported by USPHSGs DA033206, DA026746, DA033882 and P30AI082151 (the Chicago D-CFAR), and, the Daniel F & Ada L Rice Fdn. and the Rush University Center for Compulsive Behavior and Addiction.

Conditional central expression of HIV-1 Tat protein potentiates cocaine- and ethanol-mediated reward and reinstates extinguished reward-seeking behavior

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We hypothesized that HIV-1 Tat expression in brain would modulate the rewarding effects of cocaine and ethanol. Using the GT-tg bigenic mouse model, where brain-selective Tat expression is controlled by activation of a doxycycline (Dox) promotor, we tested the effects of Tat protein on cocaine- and ethanol-conditioned place preference (CPP). Western blot analysis confirmed the expression of Tat protein in GT-tg bigenic mice correlated with dose and duration of Dox treatment. In behavioral testing, although GT-tg bigenic mice expressing Tat demonstrated saline-conditioned place preferences similar to uninduced littermates and saline- or Dox-treated C57BL/6J mice, Tat expression significantly increased both cocaine- and ethanol-CPP 3-fold. Consistent with this observation, subsequent characterization found the potentiation of CPP for both rewarding drugs to be dependent on the magnitude of exposure to Tat protein. Of interest, among GT-tg bigenic mice demonstrating extinction of either cocaine- or ethanol-CPP, exposure to Tat protein subsequently resulted in the reinstatement of the extinguished place preference response in previously uninduced mice. Overall, these data suggest that exposure to HIV-1 Tat protein in mouse brain is sufficient to increase the rewarding effects of abused substances. Moreover, the Tat-induced reinstatement of an extinguished place preference for cocaine and ethanol suggests a biological means by which HIV infection may increase the vulnerability to substance abuse and relapse in abstinent subjects. Supported by NIMH R01 MH085607

Immune Activation Measures in GALT in ART Naive Methamphetamine-Using HIV Positive MSM

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Introduction: It is unknown whether high plasma viral load levels associated with use of methamphetamine (meth) among people living with HIV is related to poor ART adherence or to a direct interaction of meth and immune function. This study examined acute effects of meth use on markers of immune activation in gut associated lymphoid tissue (GALT) methamphetamine HIV-positive men who have sex with men (MSM) in Los Angeles. **Methods:** 67 MSM responded to a community-wide recruitment campaign. Of these, 8 completed all screening procedures for the study and provided a required urine sample that was positive for meth metabolites and negative

for all other drugs, documenting recent meth use and no other illicit drug use. On a second screening visit at least a week later, these 8 provided samples of rectal fluid and tissue biopsies. On the day of the second visit, 5 tested positive for recent meth; 3 tested negative for meth. Results: Findings showed that pro-inflammatory cytokines MIP-1a ($p=0.051$) and IL-1b ($p=0.053$) were significantly higher for meth-positive MSM ($n=5$) than for meth-negative MSM ($n=3$). Similarly total rectal IGG was significantly higher for meth-positive MSM than for their peers who tested negative for meth. Discussion: These findings provide an initial indication that acute methamphetamine use may cause disruption of immune system control of HIV in GALT via release of pro-inflammatory cytokines. Supported by CFAR/NIAID P30 AI28697

Symposium #5: Antiretroviral Therapy and the CNS

Antiretroviral Therapy and the Brain

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One of the most common CNS disorders that occurs in people living with HIV (PLWH) disease is HIV-associated neurocognitive disorder (HAND). Its pathogenesis is incompletely understood but is thought to result from a cascade of events that begins with migration of activated and HIV-infected lymphocytes and monocytes into the brain. Subsequent events, including glial activation and production of viral and host neurotoxins, ultimately leads to neuronal injury, the pathogenic event that appears to underpin HAND. HAND responds to antiretroviral therapy (ART), but not in all PLWH. Distribution of ART into the central nervous system (CNS) can be severely limited by the blood-brain barrier (BBB). This may result in persistent HIV replication in the CNS, as evidenced by increasing reports of CSF "viral escape", and could lead to new or persistent HAND in treated individuals. The substantial restriction exerted by the BBB also presents a challenge for HIV eradication. Better control of HIV in the brain is probably necessary "but not always sufficient" for treatment of HAND. For this reason, strategies to improve distribution of ART into the CNS may have therapeutic benefits. Such strategies would have to account for other factors that can influence recovery from HAND, including the monocyte efficacy of ART, the neurotoxicity of ART, and the presence of comorbid conditions, such as HCV disease. In summary, HAND continues to afflict PLWH despite ART, highlighting the need for better understanding of the pathogenesis, improved detection strategies, and more effective therapy. Supported by National Institute of Mental Health/K24 MH097673

Neuroprotective CCR5 Inhibition in the SIV/Macaque Model of HAND

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HIV-associated neurologic disorders (HAND) remain a significant challenge despite the efficacy of combined antiretroviral therapy (cART). Given the association between HAND and replication of HIV in macrophages in the brain, CCR5 antagonists could attenuate CNS disease by limiting HIV replication in macrophages and by dampening pro-inflammatory signaling mediated by chemokine-CCR5 interactions. To determine whether CCR5 inhibition altered CNS disease progression, SIV-infected macaques were treated solely with the CCR5 inhibitor maraviroc. SIV RNA and SIV DNA levels in brain were markedly lower in maraviroc treated, SIV-infected macaques versus untreated SIV-infected macaques, demonstrating that maraviroc monotherapy limited replication of SIV in the CNS and potentially reduced the CNS latent viral reservoir. In addition, maraviroc treatment lowered monocyte and macrophage activation represented by CNS CD68 immunostaining and plasma sCD163 levels. TNF α and CCL2 RNA expression in brain also were significantly lower with maraviroc treatment. Maraviroc also reduced axonal amyloid precursor protein (APP) immunostaining to levels present in uninfected animals, demonstrating protection from neuronal dysfunction. Although maraviroc therapy reduced plasma viral load and SIV RNA levels in spleen, relative decreases were less than CNS declines, underscoring the importance of assessing CNS-specific outcomes in evaluating efficacy of CCR5 inhibition. The addition of CCR5 inhibitors to cART may prevent neurologic disorders in HIV-infected individuals and may reduce CNS viral reservoirs. Supported by NIH HL078479

Nanomedicines and Antiretroviral Therapy

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Drug toxicities, patient regimen adherence and limited penetrance into viral reservoirs have diminished the effectiveness of long-term antiretroviral therapy (ART). Novel strategies developed in the Nebraska laboratories

have served to improve ART delivery to HIV-1 target cells including mononuclear phagocytes (MP: monocytes and tissue macrophages) and CD4+ T lymphocytes. Over the past decade we pioneered the development of long-acting injectable nanoformulated ART (nanoART) with superior pharmacokinetics. A “Trojan Horse” MP carriage of the drug formulations was developed to facilitate maintenance of long-lived storage depots including those in liver and spleen. Our drug formulations enable drug targeting to an infected CNS. We maximized viral clearance by developing “boosting”← drug-drug interactions for our protease inhibitors. A novel mixed-lineage kinase-3 inhibitor (URMC-099) with known immune and neuromodulatory activities potentiated antiretroviral activities by sustaining drug levels in MP endosomal compartments targeted for viral assembly. Reduction in residual virus was substantive seen by modulating Rab-linked proteins. Synthesis of polymer drugs with attachments of specific ligand coating provides potential synergy allowing drugs to target specific targets in tissue viral reservoirs. We also probed directed formulations in rodents using a newly discovered small magnetite ART (SMART) platform to improve pharmacokinetic screenings. Such combinations of antiretrovirals and adjunctive therapies may represent a novel means for viral clearance. Supported by 5RO1NS076386, P01 DA028555, P01 NS31492

HIV, cART and the brain

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Efforts to develop treatment strategies to eliminate HIV from the central nervous system (CNS) with new and existing antiretroviral drugs (ARVs) are hampered by incomplete information on potential neurotoxicity. To provide an initial neurotoxicity screen for current ARVs, we performed in vitro studies in which primary rat neurons were exposed for 2-7 days to relevant concentrations of reverse transcriptase, protease or entry inhibitors. Assays were designed to provide sensitive measures of morphological damage and neural dysfunction. The extent of damage was typically modest with median toxic concentrations (TC50) ranging from 2 to 10,000 ng/ml. The greatest neural damage was associated with abacavir, efavirenz, etravirine, nevirapine, and atazanavir, while the lowest was seen with darunavir, emtricitabine, tenofovir, and maraviroc. Using a calculated index of potential risk, eleven compounds showed tangible risk and five compounds minimal risk at estimated therapeutic concentrations. ARV combinations did not show additive damage and in some cases showed a trend toward less damage. ARVs did not directly affect intracellular calcium but influenced the ability of neurons to regulate calcium in response to an excitatory challenge. No relationship was seen between depolarization of mitochondria and neural damage. These data provide initial evidence to identify potential risks of antiretroviral compounds in the CNS. More information is needed on the types and mechanisms of ARV toxicity to guide the development of safe treatment options for the control of HIV in the CNS. Supported by NIH AIDS Reagent Program.

Cerebrovascular homeostasis and cognitive function: divergent effects of ART and adiponectin

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While combination antiretroviral therapy has revolutionized HIV clinical care and tempered HIV-associated morbidity and mortality, certain antiretroviral drugs, particularly protease inhibitors (PI), are frequently associated with metabolic complications including lipodystrophy, dyslipidemia, and insulin resistance. These metabolic comorbidities are well-known risk factors for cardiovascular disease, and can significantly undermine neurologic function as well. Based on previous studies highlighting the important role of lipodystrophy in driving PI-induced neurologic injury, this study was designed to determine if preservation of the adipokine adiponectin could neutralize the detrimental effects of PI treatment in experimental animals. Adult male C57BL/6 mice were subjected to a clinically relevant, 4-week regimen of lopinavir/ritonavir in conjunction with either genetic or pharmacologic adiponectin replacement therapy. Comprehensive metabolic, neurobehavioral, and biochemical analyses revealed that adiponectin therapy reversed PI-induced loss of subcutaneous adipose tissue, attenuated PI-induced hyperinsulinemia and hypertriglyceridemia, and prevented PI-induced cognitive impairment and brain injury. Further analyses suggest that the protective effects of adiponectin are based on preservation of cerebrovascular homeostasis. Collectively, these data reinforce the link between metabolic co-morbidities and cognitive impairment and suggest that pharmacological reactivation of adiponectin pathways could remediate key aspects of PI-induced metabolic syndrome in clinical settings. Supported by NIH (MH099944)

Symposium #6: Neurogenesis and Repair

CXCL12 chemokine/CXCR4 chemokine receptor-signaling in the hippocampal stem cell niche supports neurogenesis and positive reinforcement learning.

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CXCL12/CXCR4 signaling guides migration of immune cells and neuronal precursors. The second CXCL12 receptor, CXCR7, regulates CXCR4 signaling by controlling CXCL12 availability. We examined this system in the adult brain using immune- and in situ hybridization histochemistry, transgenic reporter mice and inducible conditional knockout (icKO) mice. This revealed widespread expression of Cxcr7 and Cxcl12 in microvessels and neurons as well as a highly restricted Cxcr4 signal that was most prominent in neurogenic regions. In the dentate gyrus (DG), neural stem cells (NSCs), neuronal-committed progenitors and immature neurons but not mature granule neurons (GNs) expressed Cxcr4. Cxcr7 was seen in GNs and NSCs of the DG while Cxcl12 was abundant in GNs. Pharmacological approaches and immunodetection of CXCR4 were combined in vivo and in vitro and showed that endogenous CXCL12 causes tonic activation of CXCR4 in hippocampal NSCs. Cxcr4-icKO in NSCs reduced neurogenesis and evoked ectopic placement of new neurons whereas Cxcr7-icKO in NSCs did not affect neurogenesis but caused ectopia of new neurons. A CXCR4 antagonist impaired long-term memory in a neurogenesis-dependent task and Cxcr4-icKO impaired shuttle box learning in a paradigm that used electrical stimulation of the reward system for positive reinforcement. Thus, we established CXCL12 as essential paracrine factor in the hippocampal stem cell niche. CXCL12/CXCR4-dependent hippocampal neurogenesis is required to associate a normally neutral stimulus with a rewarding stimulus during learning of a conditioned response. Supported by German Research Foundation (STU295/5-1).

In vivo reprogramming of reactive astrocytes for neural regeneration

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Injuries to the central nervous system lead to irreversible neuronal loss. Neural regeneration from endogenous cells could be an ideal approach to replenish the lost neurons and repair the damage. Astroglia is a hallmark of neural damage. These reactive astrocytes are initially beneficial by restricting damage spread but detrimental for long-term recovery and repair of the injured central nervous system. We examined ways to change the fate of these astrocytes to neurons by in vivo screens in the adult central nervous system. Through multiple genetic lineage-tracing approaches, we show that a single transcription factor is sufficient to reprogram resident mature astrocytes into proliferative neuronal precursors in the adult mouse brain or spinal cord. These induced adult neuronal precursors (iANPs) persist for months and can be generated even in aged mice. When supplied with neurotrophic factors or treated with a small molecule, iANPs develop into electrophysiologically mature neurons, which functionally integrate into the local neural network. Our results demonstrate that adult reactive astrocytes can be reprogrammed to show remarkable plasticity in vivo, a feature that might have important implications in the regeneration of the central nervous system using endogenous patient-specific glial cells. Supported by NIH: 1DP2OD006484 and R01NS070981.

Regulation of neurogenesis by store operated CRAC channels

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Calcium signals regulate many critical processes during vertebrate brain development, including proliferation and differentiation of neural stem cells (NSCs), neurotransmitter specification, and axonal outgrowth. Yet the identity of the ion channels mediating Ca²⁺ signaling in NSCs is unclear. Here, we find that embryonic and adult mouse NSCs exhibit store-operated calcium entry (SOCE) mediated by Ca²⁺ release-activated Ca²⁺ (CRAC) channels. SOCE in NSCs was blocked by CRAC channel inhibitors, and Western blot analysis revealed expression of STIM1 and Orai1. Knock down of STIM1 or Orai1 significantly diminished SOCE in NSCs, and SOCE was lost in NSCs from transgenic mice lacking Orai1 or STIM1. Thus, STIM1 and Orai1 make essential contributions to SOCE in NSCs. SOCE in NSCs was activated by the mitogens and neurotransmitters including epidermal growth factor and acetylcholine, the latter occurring through muscarinic receptors. Activation of SOCE resulted in gene transcription through calcineurin/NFAT signaling. In addition, suppression or deletion of STIM1 and Orai1 expression significantly attenuated proliferation of both embryonic and adult NSCs, both in NSCs cultured as neurospheres and in vivo, in the sub-ventricular zone of adult mice. These findings indicate that CRAC channels serve as a major route of Ca²⁺ entry in NSCs and regulate key effector functions in NSCs including gene-expression and proliferation, portending several ways in which this Ca²⁺ entry mechanism could contribute to vertebrate brain development. Supported by NIH/NS057499.

Selective targeting of neural progenitors by HIV-1 and opiates throughout ontogeny

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HIV and/or substance abuse can affect the genesis of neural progenitors. Deficits in neurogenesis have been associated with neurocognitive impairment. We hypothesized that neuronal/glial progenitors (NPC/GPCs) are targets of HIV proteins or HIV infection. We have also explored whether opioid drugs exacerbate such effects. In ongoing studies, NPCs were found to be targets of HIV, but the effects are limited to specific developmental periods and/or stages of differentiation, and cell lineage may be a key determinant in vulnerability. For example, treatment with Tat or infectious R5-tropic HIV supernatant (HIV) decreased proliferation of Sox2+ (multipotent neural) precursors and Olig2+ (pre-oligodendrocyte [OL]) progenitors in a time-dependent manner, but had minimal effect on nestin+ cells. While morphine co-treatment accelerated/increased effects of Tat or HIV on DNA synthesis in Sox2+ NPCs, Olig2+ OLs were unaffected, also suggesting stage and lineage specificity as factors in HIV and mu opioid receptor agonist interactions. HIV and morphine induced NPC/GPC death in A2B5+ and immature Olig2+ OLs, but not in nestin+ or Sox2+ NPCs, or mature OLs and astroglia. In vivo, the proliferation of NPC/GPCs is reduced in neonatal mice by Tat/morphine exposure, with concomitant changes in specific cell populations, which were similarly reduced after 3 mo Tat exposure in adult mice. Overall, selective HIV +/- opiate effects on NPC/GPCs and their progeny likely distort the normal balance of neuronal and glial populations. Such numeric or phenotypic mismatching likely alters CNS function. Supported by NIH NIDA DA027374, DA024461, NS069216.

Laboratory tools using neural progenitor cells to study neurovirulent viruses with restricted host range for the human brain

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Many neurovirulent viruses target specific cells in the nervous system which makes investigations of pathogenesis more difficult than other organ systems. Both HIV-1 and JC Virus are examples of host range restrictions or tropism for human glial cells, astrocytes and oligodendrocytes. Consequently, new technologies needed to be developed to study these viruses to gain insight into biological and molecular mechanisms of infection. We have established a unique cell culture model of the human brain through identifying, isolating and propagating human neural progenitor cells from which neurons, astrocytes and oligodendrocytes can be derived in pure populations. We identified the progenitor cells using a specific anti-nestin antibody produced for this work that identifies neural stem/progenitor cells that does not cross react to other species. The human brain derived progenitor cells can be differentiated to neuronal or glial phenotypes that show increased susceptibility to infection. Measuring cellular and viral gene expression in this model of infection during the differentiation process allows identification of specific gene sets that not only allow viral infection but also plays a role in maturation of cell types in the human brain.

Special Session Late Breaking SNIP Research

Putting the PINCH on Neurodegeneration in HIV

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Several studies report associations between the PINCH (particularly interesting new cysteine histidine-rich) protein and HIV-associated CNS disease. PINCH is detected in the CSF of HIV patients and levels may be indicative of changes in disease status over time. PINCH binds hyperphosphorylated Tau (hpTau) in the brain and CSF, but little is known about the relevance of these interactions to HIV CNS disease. In this study, PINCH and hpTau levels were assessed in three separate CSF samples collected longitudinally from 20 HIV+ patients at intervals of approximately 1, and 6 months from the initial visit (baseline). Correlational analyses were conducted for CSF levels of PINCH and hpTau and other variables including plasma CD4+ T-cell count, plasma and CSF viral burden, CSF neopterin, white blood cell (WBC) count, and antiretroviral CNS penetration-effectiveness (CPE). Statistical analyses showed that the fold-change in CSF PINCH protein levels were significantly higher in patients with CD4 counts <200 cells/mm³ compared to those with CD4 counts Supported by NIMH

Hepatitis C Virus Interaction with Astrocytes and Microglia

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Hepatitis C virus (HCV) infection causes the central nervous system (CNS) abnormalities in more than 50% of chronically infected subjects. However, the underlying mechanisms are largely unknown. Thus, we characterized the HCV interactions with astrocytes and microglia, two putative HCV target cells in the CNS. Primary human astrocytes (PHA) and microglia were very inefficiently infected by HCV, either in the cell-free form or through cell-cell contact, although PHA expressed all known HCV receptors. HCV IRES-mediated RNA translation was functional in both cells and further enhanced by miR122 expression. Nevertheless, PHA did not support HCV replication regardless of miR122 expression. To our great surprise, HCV exposure induced robust IL-18 expression in PHA and microglia and exhibited direct neurotoxicity. Taken together, these results showed that astrocytes and microglia did not support productive HCV infection and replication, but HCV interactions with astrocytes/microglia and neurons alone might be sufficient to cause CNS dysfunction.

Symposium #7: Neuropathic pain / Neuroimmune interactions in the modulation of pain

Inhibition of inflammatory and neuropathic pain by immune cell-derived opioids

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The concept that the immune system can communicate with peripheral sensory neurons to modulate pain is mostly based on documented interactions between opioid ligands and receptors. Such findings may have broad implications for the development of safer pain medication. Innovative strategies take into account that analgesics should be particularly active in pathological states rather than producing a general suppression of the central nervous system, as with conventional morphine- or cannabinoid-like drugs. Nerve injury or inflammation of peripheral tissue leads to increased functionality of opioid receptors on peripheral sensory neurons and to local production of endogenous opioid peptides in immune cells. Endocannabinoids were also detected in leukocytes but their role in pain modulation is yet to be addressed. Future aims include the development of peripherally restricted opioid agonists, selective targeting of opioid-containing immune cells to sites of painful injury and the augmentation of peripheral ligand and receptor synthesis, e.g. by gene therapy. Similar approaches may be pursued for cannabinoids. The ultimate goal is to avoid detrimental side effects of currently available analgesics such as respiratory depression, cognitive impairment, addiction, gastrointestinal bleeding and thromboembolic complications. This presentation will discuss basic and clinical data demonstrating that peripherally active exogenous and endogenous opioids can potentially inhibit neuropathic and inflammatory pain. Supported by EU FP7-HEALTH-2013-INNOVATION-1; No. 602891-2

Protection of neuropathic pain by pro-resolution lipid mediators

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Neuropathic pain is a debilitating syndrome associated with pathological changes, such as neuroinflammation and neuronal hyperactivity, in the peripheral and central nervous system. Omega-3 unsaturated fatty acids docosahexaenoic acid (DHA) and eicosapentaenoic acid (EPA), derived from fish oil, are known to have beneficial effects including analgesic effects. However, the analgesic effects of resolvins and protectins, the metabolic products of DHA and EPA, are much more potent than their precursors. Resolvins, such as resolvin E1 (RvE1), and protectin D1 (PD1/NPD1) represent a new class of pro-resolution lipid mediators (PRLMs). RvE1 and NPD1 potently inhibit inflammation and inflammatory pain. Recently, we also found that RvE1 attenuated neuropathic pain via inhibiting microglial signaling. Interestingly, NPD1 is more effective than RvE1 for treating neuropathic pain. Peri-surgical treatment of NPD1 prevents nerve injury-induced mechanical allodynia and ongoing pain in mice. Intrathecal post-treatment of NPD1 also effectively reduces established neuropathic pain. Mechanistically, NPD1 treatment blocks nerve injury-induced long-term potentiation, neuroinflammation, and reverses synaptic plasticity in the spinal cord. Collectively, PRLMs and related mimetics might serve as a new class of analgesics for preventing and treating neuropathic pain. Supported by NIH grants DE17794, DE22743, NS82985, and NS67686.

Modulation of non-classical opioid receptors and control of neuropathic pain

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Accumulating evidence indicates that opioids induce Toll-like receptor 4 (TLR4)-mediated neuroinflammatory responses by glia and primary afferent sensory neurons (SN) including opioid-induced hyperalgesia. To diminish this effect of opioids on TLR4 in sensory neurons and enhance the efficacy of this class of drugs for neuropathic pain states we pharmacologically targeted neuronal TLR4-mediated increases in the voltage-gated sodium current Nav1.7 (encoded by SCN9A). Nav1.7 is a predominant tetrodotoxin-sensitive Na⁺ channel which amplifies small subthreshold depolarizations and regulates neuronal excitability. A known stabilizer of the inactivated state of Nav1.7, carbamazepine (CBZ), was evaluated for its ability to diminish drug-induced increases in neuronal TLR4 excitation. We demonstrate that increased SN excitation which follows exposure to the known TLR4 agonist, morphine-3-glucuronide (M3G), is CBZ-sensitive. Next we determined whether CBZ could potentiate the effects of morphine (MOR) in a rodent model of neuropathic pain; tibial nerve injury (TNI). MOR (5 mg/kg, i.p) exerted antinociceptive effects at postinjury days 7 and 14, but not day 21 or 28. CBZ alone (10 mg/kg, i.p.) failed to produce antinociceptive effects at any time following TNI (days 7-28). In contrast, coadministration of 1 or 5 mg/kg MOR with 10 mg/kg CBZ reversed tactile hyperalgesia at post-injury day 28 when compared with either drug alone. Taken together, these data suggest that the combination of MOR and CBZ can achieve greater efficacy and opioid-sparing in a model of neuropathic pain. Supported by NIH/NIDA

Multifunctional compounds to enhance analgesic efficacy and reduce unwanted side effects

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In the total cancer patient population, bone pain is the single most commonly reported complaint. Tumor manifestation in bone disrupts normal bone cell metabolism, leading to skeletal-related events, spontaneous fracture and other bone-related pathologies. In addition to painful fractures and remodeling, bone tumors are a source of pro-inflammatory cytokines, chemokines, acidity and algogenic substances such as glutamate which are free to act at local nerve endings yet, pro-nociceptive signaling initiated by bone metastases is poorly understood. It is ongoing pain in cancer metastases that is clinically targeted by high doses of opioids. However, moderate-to-severe cancer pain is not adequately managed in >50% of patients and has recently been demonstrated as enhancing bone wasting in our model of bone cancer. In contrast, cannabinoid receptor 2 (CB2) receptor specific agonists have been shown to reduce bone loss, stimulate bone formation in a model of osteoporosis and produce analgesia in both inflammatory and neuropathic pain models. Here we demonstrate for the first time using a syngenic mouse model of breast-induced bone cancer that CB2 agonists (JWH015 6mpk for 7days) reduce breast cancer-induced bone pain, reduce bone wasting while decreasing tumor-induced osteoclast activity, and significantly reduces breast cancer proliferation within the bone via cytokine/chemokine suppression. In addition, preliminary studies on how morphine may act via a non-traditional opioid receptor to promote bone loss in a model of cancer pain will be presented. Overall, CB₂ receptors are an important molecular target in breast cancer-induced bone pain with hopes of establishing a novel aim in which drugs may better manage pain for patients with bone metastases.

Poster Abstracts By Session

Abstracts submitted for ECITA Poster Session 1

Determination of HIV-1 X4 and R5 genetic signatures within the LTR, Tat and Vpr

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During the course of human immunodeficiency virus type 1 (HIV-1) infection, viral adaptation involves genetic alterations across the genome with genetic signatures preferentially associated with viral phenotypes which can be categorized based on the co-receptor usage that are CCR5-utilizing (R5), CXCR4-utilizing (X4), and the dual tropic (X4R5) viruses. The envelope-V3 (Env-V3) sequence is known as a major co-receptor usage determinant, which can be predicted by different in silico methodologies. Utilizing the position-specific scoring matrix (PSSM) algorithm, Env-V3 sequences derived from the Los Alamos National Laboratory (LANL) database and DREXELMED HIV/AIDS Genetic Analysis cohort (DM), can be classified into X4 and R5 genotypes, with associated specific co-linear long terminal repeat (LTR), Tat, and Vpr, genotypic patterns. Differential amino acid (DAA) signatures in both Tat and Vpr, including differential nucleotide (DN) signatures in the viral LTR were

identified between the R5 and X4 viruses. Utilizing a maximum likelihood strategy, genetic relatedness of Env-V3 between X4 and R5 was readily characterized, whereas, those of Tat, Vpr and LTR were not. Conversely, genetic diversities of all genes/regions of interest determined by the mean genetic distance (MGD) were evaluated between the group of X4 and R5, indicating the differential evolution of viral genes. These differential signatures will be further evaluated to determine if selective pressures like drug of abuse affect the X4/R5 evolution and HIV-1-associated disease progression. Supported by This work is supported by NIH/NINDS R01 NS32092, NIDA R01 DA19807, NIMH P30 MH092177, and NIMH T32 MH079785

Morphine and HIV infection additively inhibits phagocytosis in a murine model of HIV

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We have shown in previous studies that morphine increases susceptibility to opportunistic infections by impairment in phagocytosis. In the current study, we determined the effects of EcoHIV and morphine together on phagocytosis. EcoHIV was developed to simulate HIV infection pathogenesis by genetically modifying HIV to infect mouse cells by substituting gp80 from Murine Leukemia Virus for gp120 of HIV. Our results show a significant decrease in phagocytosis of opsonized FITC dextran bead in mouse peritoneal macrophages in EcoHIV infected morphine treated mice (41.2 ± 1.6 , $p=0.029$) when compared to placebo. The inhibition of phagocytosis was higher than the effects of morphine ($64.6 \pm 8.2\%$, $p=0.03$) and EcoHIV ($56.8 \pm 4.1\%$, $p=0.01$) alone. Similarly, in vitro studies using RAW 264.7 and J774 A.1 mouse macrophage cell lines showed that morphine reduced phagocytosis in EcoHIV infected mice ($49.8 \pm 8.3\%$, RAW264.1 & $52.2 \pm 6.1\%$ J774A.1) and the effects were higher than the inhibition of phagocytosis by EcoHIV ($71.9 \pm 14.3\%$ RAW264.1 & $70.0 \pm 15.4\%$ J774A.1) or morphine ($71.8 \pm 3.9\%$ RAW264.1 & $74.5 \pm 7.1\%$ J774A.1) alone. The p24 levels were significantly higher in the morphine infected J774 A.1 macrophages, 2 days post- infection compared to EcoHIV alone. These results suggest that synergistic effects of morphine and EcoHIV in inhibiting phagocytosis may be a major contributing factor underlying increased infections in opioid addicted HIV population. The additive effects of EcoHIV and morphine on bacterial killing and clearance; and inhibition of the actin polymerization is currently under investigation. Supported by RO1 DA 12104, RO1 DA 022935, RO1 DA031202, K05DA033881, P50 DA 011806, 1R01DA034582

Effect of mild-to-moderate smoking on viral load and oxidative stress in HIV-1-infected individuals: Role of cytochrome P450 enzymes

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Mild-to-moderate tobacco smoking is highly prevalent in HIV-1-infected individuals, and is known to exacerbate HIV-1 pathogenesis. The objective of our *ex-vivo* study was to determine the specific effects of smoking on viral load and oxidative stress in ART naïve HIV-1-infected individuals, and delineate the role of cytochrome P450 (CYP) pathway in these pathogenic effects. We recruited human subjects from four cohorts: healthy, HIV-1-infected, smokers, and HIV-1/smokers in Cameroon, Africa using strict exclusion criteria. Upon recruitment of these subjects, we collected the blood samples and separated plasma and monocytes. Our results showed a significant increase in viral load in plasma and oxidative DNA damage in both plasma and monocytes of HIV-1/smokers compared to all other cohorts. Further, the results showed a significant increase in nicotine metabolism in HIV-1/smokers compared to smokers alone. Although an increase in viral load and oxidative stress correlated with increased nicotine metabolism and decreased AOE, it did not correlate with CYP2A6 level in HIV-1/smokers compared to smokers. Nevertheless, we have earlier the role of CYP2A6 in nicotine metabolism and ROS production in U937 cells. Taken together, these results show a correlation of CYP2A6-mediated nicotine metabolism with oxidative DNA damage and viral replication. We are currently performing an in-vitro study using cigarette smoke condensate (CSC) in order to further dissect the mechanisms by which CYP-regulated pathways contribute to smoking-mediated oxidative damage and cell death in U937 monocytes. Our recent results showed higher levels of reactive oxygen species in CSC treated cells than control. We also found an increased levels of CYP1A1 and CYP2A6 at both mRNA and protein level upon treatment with CSC. Our future studies are to: 1) determine CSC-mediated toxicity by measuring cleaved caspase-3 and annexin-PE staining, 2) dissect molecular mechanisms involved in CSC-mediated induction of both CYP1A1 and CYP2A6, and 3) examine the role of CYPs in CSC-mediated ROS production and subsequent toxicity by using specific CYP inhibitors and antioxidants.

Tyrosine hydroxylase expression in the substantia nigra of MPTP-treated mice is increased in GABAergic neurons by chronic administration of dopamine replacement compounds L-DOPA and BL-1023

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We previously showed that chronic administration for 35 days of L-3,4-dihydroxyphenylalanine (L-DOPA) or BL-1023 (an L-DOPA-GABA conjoined compound) improved locomotor function and increased numbers of tyrosine hydroxylase (TH) immunoreactive (TH+) neurons in the substantia nigra (SN) of 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine (MPTP)-treated mice compared to those given no drug. We hypothesized increased numbers of TH+ neurons are derived from a phenotypic shift of GABAergic neurons that upregulate TH, or alternatively were products of dopaminergic neurogenesis. To test the former, we analyzed ventral midbrain tissue sections for expression of TH and glutamic acid decarboxylase-67 (GAD67), a marker for GABAergic neurons. Stereological analysis demonstrated that mice treated with either L-DOPA or BL-1023 showed significant increases in the numbers of TH+GAD67+ neurons in the substantia nigra compared to controls. Stereological analysis revealed no significant nuclear incorporation of the thymidine analog (BrdU) by TH+ neurons within the SN of mice suggesting little or no neurogenic activity. These findings support the notion that numbers of GAD67+ neurons upregulate TH expression and effectively increase the numbers of TH+ neurons in response to MPTP-intoxication, loss of dopaminergic neurons, and chronic administration of dopamine-replacement drugs. Furthermore, these findings suggest that the upregulation of TH expression by GABAergic neurons reflect a putative compensatory or reparative mechanism to increase dopamine production in a dopamine-depleted environment. Supported by UNMC Fellowship (KMA) and NIH grant R01NS070190 (RLM)

HIV-1 Tat genetic variation correlates with associated neurocognitive impairment and demonstrates compartmentalization

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The current studies seek to identify and characterize genetic sequence variation within the HIV-1 protein Tat on the basis of neurocognitive impairment, anatomical source, and patient cohort. HIV-1 Tat sequences were obtained from both the DREXELMED HIV/AIDS Genetic Analysis Cohort as well as from autopsy brain tissue regions obtained from the National NeuroAIDS Tissue Consortium (NNTC). Sequences acquired from the DREXELMED cohort were amplified from peripheral blood mononuclear cells (PBMCs), while NNTC samples were amplified from six regions of the brain (cerebellum, deep white matter, head of caudate, mid-frontal gyrus, parietal lobe, and thalamus) in addition to the spleen. Tat nucleotide sequences were translated and aligned to the consensus subtype B (ConB; Jan 2002) HIV-1 reference genome in order to compare sequence similarity across anatomical compartments and degree of neurocognitive impairment. Aligned sequence populations were also compared across all positions of Tat on the basis of amino acid composition and similarity using amino acid substitution matrices. Multiple positional hotspots were identified as being statistically different between populations, including positions 74 and 100. In contrast, the TAR-binding domain was shown to be highly conserved. Overall, these analyses have resulted in the identification of compartmentalization between brain and PBMC-derived sequences, as well as Tat sequence variants specific for patients with or without neurocognitive impairment which may prove useful in further characterization of HIV-1 pathogenesis.

Role of HDAC2 and miR-485 in regulation of synaptic plasticity genes in HIV infection: Implication in HAND

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HIV-associated neurocognitive disorders (HAND) remain as an important problem in HIV-infected individuals despite the use of combination antiretroviral therapy. The main cognitive deficits observed in HAND involve attention and working memory, executive functioning and speed of informational processing. The process of learning and memory consolidation requires synaptic plasticity characterized by physical changes to, and gene expression changes in, neuronal synapses. We hypothesize that increased levels of HDAC2 inhibits the transcriptional process while elevated levels of miR-485 negatively regulate the synaptic plasticity and spine density in HIV infected neuronal cells. In this study, we have examined the HDAC2 expression, levels of miR-485

and expression of human synaptic plasticity genes in HIV infected SK-N-MC neuronal cells. Results showed up-regulation of HDAC2 and elevated levels of miR-485 in HIV infected SK-N-MC cells. Further, using human synaptic plasticity PCR array, out of 83 synaptic plasticity genes analyzed, 26 genes were significant down-regulation in HIV infected SK-N-MC cells. We have also observed decreased spine density in HIV infected SK-N-MC cells. In addition, inhibition of HDAC2 up-regulation with the use of vorinostat and inhibition of miR-485 using anti-miR-485 resulted in recovery of synaptic plasticity genes expression and spine density in HIV-infected SK-N-MC cells. Our results indicate that increased levels of HDAC2 and miR-485 in HIV infection negatively regulate the synaptic plasticity genes expression and spine density. Supported by 5R01DA021537 and 1R037DA025576

Induction of ER stress and dysregulation of autophagy in cells of the blood brain barrier by anti-retroviral drugs.

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The profile of HIV infection has drastically changed since the introduction of anti-retroviral drugs (ARVd); however long-term survivors frequently develop a slowly progressing neurodegenerative disease. We hypothesise that the side effects of the drugs used in HIV treatment contribute to this observed neuro-pathology. We exposed brain endothelial cells and astrocytes to the combination of antiretroviral drugs used to initiate treatment, either Efavirenz, Tenofovir and Emtricitabine (cART1) or Lamivudine, Indinavir and Emtricitabine (cART2), and evaluated their impact on known cellular stress pathways. In cells treated with cART1, we observed a dramatic induction of the ER stress pathway resulting in increased expression of ER stress sensors and effectors, such as IRE1 α , CHOP, and BIP. Surprisingly, these effects were accompanied by a significant reduction in autophagy activity by analysing autophagic vacuole processivity and LC3b cleavage. In subsequent studies, we identified that Efavirenz was responsible for the observed effects and that the toxicity was in part reversible. These results indicate that side effects of the ARVd drug Efavirenz include induction of ER stress and dysregulation of the autophagy pathway, which may contribute to the disruption of the blood-brain barrier observed in HIV patients. In conclusion, care should be taken in the choice of ARVd to prevent toxicity that could contribute to cerebrovascular pathology associated with HIV treatment. Supported by This study was supported by MH098891, MH072567, MH063022, and DA027569.

Chronic morphine decreases gp120BaL induced cell death in the rat striatum through the release of CCL5.

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The effect of opioids in the progression of HIV-associated Neurocognitive Disorders (HAND) remains under contention. CCR5 preferring strains of HIV are associated with primary infection, thereby initiating the damaging effects of HIV in both the periphery and the brain. Moreover, it has been established that the HIV protein gp120BaL alone can cause direct neuronal apoptosis by binding to CCR5. Therefore, studying the effects of opioids on gp120BaL toxicity may give insights to the pathogenesis of HAND. In this study, rats were stereotaxically injected with gp120BaL (400ng) into the striatum, and the effect of chronic morphine or morphine withdrawal on neuronal loss was determined by caspase-3, FluoroJade, and TUNEL immunoreactivity. Rats undergoing chronic morphine treatment (10mg/kg escalating to 30mg/kg b.i.d) showed a decrease in caspase-3 and FluoroJade immunoreactivity compared to saline. Conversely, animals undergoing morphine withdrawal exhibited increases in caspase-3 and FluoroJade. Morphine is known to elicit the production of CCL5, a natural ligand to CCR5, in astrocytes. To determine the role of CCL5 in morphine mediated neuroprotection, animals were injected with either lentiviral vectors overexpressing CCL5, or expressing CCL5 shRNA. Overexpressing CCL5 decreased caspase-3 and TUNEL immunoreactivity, recreating the protective effect of morphine. CCL5 shRNA abolished morphine's protective effect against gp120BaL. These data suggest that the formation of withdrawal, rather than the chronic use of opioids, may contribute to the pathogenesis of HAND. Supported by NIDA/1F31DA032282

Expression of cathepsin B interactome in post-mortem brain from HIV-associated dementia patients

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Human Immunodeficiency Virus (HIV) targets CD4+T lymphocytes and cells of monocytic lineage such as macrophages and microglia. Early in the infection, the blood brain barrier is compromised allowing entry of HIV-

infected macrophages into central nervous system, triggering the development of HIV-associated neurocognitive disorders. We have demonstrated that HIV-infected blood monocyte-derived macrophages secrete the lysosomal protease cathepsin B, which contributes to neuronal death. To elucidate the mechanism of cathepsin B-induced neuronal death, we determined the macrophage-derived cathepsin B extracellular interactome by proteomics and validated the results by immunofluorescence of post-mortem brain tissue samples from the frontal white matter provided by the National NeuroAIDS Tissue Consortium (NNTC). We observed that cathepsin B interaction with matrix metalloprotease 9 (MMP9) is decreased in the supernatants of HIV-infected macrophages, while the interaction with serum amyloid component (SAPC) is increased. SAPC was over-expressed in HIV-dementia brain tissues. MMP9 immunolabeling was higher in uninfected tissue than in HIV-dementia post-mortem tissue. Cathepsin B and macrophage/microglia markers were increased in HIV-positive tissues from patients with normal cognition, and higher in HIV-dementia tissues compared to uninfected controls. The increased cathepsin B, together with decreased interaction with MMP9 may facilitate brain tissue damage, while increased interaction with SAPC may promote amyloid deposition in patients with HIV. More proteins are under study. Supported by R01MH083516 (L.M.M.), U54NS043011, R25GM061838 (Y.C.), SNRP-NINDS-1-U5, INBRE P20RR016470-12, NIMHHD 8G12-MD007600, NNTC.

Effect of Cocaine on Autophagy in SVGA Astrocytes

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Cocaine is a commonly used drug of abuse. Previous studies have revealed that cocaine can cause neuropathological dysfunction or damage in human brain. Autophagy is an intracellular activity, which is known to eliminate dysfunctional organelles during deprivation condition to protect cells from apoptotic cell death. In addition to its protective role, autophagy could also be destructive leading to cell death (autophagic cell death). This study is designed to investigate whether cocaine induces autophagy in SVGA astrocytic cell line. To determine the effect of cocaine on autophagy we measured autophagic marker protein LC3II using western blot analysis. The results showed that cocaine causes increase in LC3II level in dose- and time-dependent manner, with the peak at 1mM cocaine at 6 hours. This result was also confirmed by detecting LC3II in SVGA using confocal microscopy. Next, we sought to explore the mechanism by which cocaine induces autophagy. We found that p-mTOR and p-Bcl-2/Beclin-1 are involved in cocaine-mediated autophagy. To further delineate the involvement of this pathway in cocaine-mediated autophagy and/or cell death, we are now in the process of using selective inhibitors and siRNA of signaling proteins of this pathway. This study is novel and has clinical relevance because cocaine use is prevalent, especially in HIV-infected populations, and show exacerbated neuronal damage. Supported by National Institute of Drug Abuse grants DA025528 and DA025011

Expression of CB2 – A dynamic feature of B cells

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We recently reported that T cells and B cells exhibit very different expression patterns for type 2 cannabinoid receptors (Castaneda JT et al, JNIP 2013;8(1):323-332). While B cells expressed CB2 at the cell membrane and at intracellular sites, T cells only expressed CB2 at an intracellular location. In addition, exposing B cells to a cannabinoid in vitro promoted receptor internalization, suggesting a dynamic interaction between extracellular and intracellular receptors. We hypothesize that CB2 expression patterns may vary depending upon local tissue environment and state of differentiation/activation. A monoclonal anti-CB2 antibody and multi-parameter flow cytometry were used to assess CB2 protein expression by B cells obtained from peripheral blood, tonsils, and B cell malignancies. Both mature naïve B cells (CD20+, IgD+, CD27-) and activated B cells (CD20+, IgD-, CD27+) obtained from peripheral blood exhibited a uniform phenotype with high intracellular and extracellular CB2 expression. While tonsillar B cells exhibited CB2 at both intracellular and extracellular locations, mature naïve tonsillar B cells reproducibly expressed higher levels of CB2 than activated tonsillar B cells. In contrast, malignant B cell lines exhibited high intracellular CB2 in the absence of detectible cell surface receptor. In conclusion, it appears that both expression levels and distribution patterns for CB2 may vary with respect to cell location, differentiation, and activation. The role of these differences in CB2 expression on cannabinoid signaling and function remain to be determined. Supported by NIH/NIDA Grants #R21-DA021813 and R01-DA03018

Renin Enhances HIV replication in T cells: Role of Pro-renin Receptor & Proteolytic Activity

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Background: Recently, we demonstrated that HIV-infected T cells (TCs) are in a high renin state (JLB 2013). We now hypothesized that HIV-induces high renin state to promote its own replication. Methods: Freshly isolated human CD4+T cells (TCs) were pulsed with HIV-1HT99 and incubated in media containing renin (0, 0.1, 1.0, 5.0 nM). Dose response effect of renin assayed by p24 content of media & cellular mRNA expression for Gag & LTR. TCs transfected with VDR-siRNA were assayed for HIV replication. To confirm the role of renin & pro-renin receptors (P)RR, TCs silenced for renin or PRR followed by pulsed with HIV & then evaluated for HIV replication. Effects of both renin & HIV protease (PR) were evaluated on cleavage of AGT & Gag-polypeptides. Immunoprecipitation assay was carried out on renin; PR & HIV treated T cells to see the activation of (P)RR & promyelocytic zinc finger protein (PLZF). Cells treated under similar conditions were also evaluated for the activation of PI-3K & NFkB pathway by WB and gel shift assay. Gel shift assay was carried out in cells lacking either renin or PPR with/without HIV milieu. Results: Renin enhanced HIV replication in a dose dependent manner. PRR/ renin deficient TCs displayed attenuated HIV replication & NF-kB activation. TCs treated with renin/PR displayed dissociation of (P)RR to PLZF. Both renin & PR cleaved Agt to AngI and Gag polypeptides to p24. Furthermore, aliskiren, a renin inhibitor, inhibited renin- & PR-induced cleavage of Agt & Gag polypeptides. Conclusion: PRR activation & protease activity of Renin contributes to HIV replication

CXCR7 Is Essential for Neural Progenitor Cell Migration.

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Neural progenitor cell (NPC) migration is an essential process for both proper brain development and neuroregeneration after brain injury. Although Stromal-derived factor-1 (SDF-1, CXCL12) and its traditional receptor CXCR4 are well known to regulate NPC migration, recent studies have revealed important functions of a new receptor CXCR7. Using CXCR7-EGFP transcriptional reporter mice, we demonstrated that CXCR7 was highly expressed in NPC in the dentate gyrus. Since NPC migration is an important event in adult neurogenesis in dentate gyrus, we used in vitro NPC cultures derived from CXCR4 knockout and wild type mice to determine CXCR7-mediated NPC migration. NPCs derived from CXCR4 knockout mice migrated to CXCL12 gradient, suggesting the CXCR7 also mediates NPC migration. Addition of CXCR7 antagonist to the CXCR4 knockout NPCs completely blocked the migration, confirming that CXCR7 mediates NPC migration. To further determine the mechanism of CXCR7-mediated migration, we turned to Erk1/2 pathway. Through Western blotting, we demonstrated that CXCL12 increased Erk1/2 phosphorylation in CXCR4 knockout NPCs, suggesting that CXCR7 can activate Erk1/2. Furthermore, Erk1/2 inhibitor blocked CXCL12-mediated migration in CXCR4 knockout NPCs, suggesting Erk1/2 activation is essential for CXCR7-mediated NPC migration. Together these results reveal an essential role of CXCR7 for CXCL12-mediated NPC migration that will be important to understand neurogenesis during development and neural repair after brain injury. Supported by National Institutes of Health: R01 NS 41858-01, R01 NS 061642-01, and P01 NS043985 (JZ).

Methamphetamine (METH) regulates astrocyte excitatory amino acid transporter-2 (EAAT-2) via activation of trace amine associated receptor (TAAR1) and downstream cAMP signaling

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Glutamate is an excitatory neurotransmitter that is highly regulated in the central nervous system (CNS). High concentrations of extracellular glutamate result in excitotoxicity and can exacerbate neurodegenerative disorders, including human immunodeficiency virus-1 (HIV-1)-associated neurocognitive disorders (HAND). Additionally, drugs of abuse such as methamphetamine (METH) can increase the severity of excitotoxicity and can accelerate HAND. Excitatory amino acid transporter-2 (EAAT-2) is responsible for approximately 90% of extracellular glutamate uptake from the synapse and is primarily localized in astrocytes. Dysregulation of EAAT-2 leads to astrocytes decreased ability to clear glutamate. It is established that METH leads to excitotoxicity in neurons, however, in astrocytes the molecular mechanisms resulting in METH-mediated EAAT-2 dysregulation are unclear. Previously we showed that HIV-1ADA, METH and transient hyperthermia regulates localization and expression of astrocyte trace amine associated receptor 1 (TAAR1). Our data shows METH-induced activation of astrocyte TAAR1 increases intracellular cAMP levels in astrocytes that is significantly decreased in siTAAR1-transfected astrocytes. Further, METH treatment downregulates EAAT-2 mRNA levels. We propose downstream cAMP signaling pathways of METH-induced astrocyte TAAR1 activation result in EAAT-2 dysregulation. The results of

this study will uncover novel molecular mechanism of METH-induced astrocyte TAAR1 activation and the downstream effects of cAMP signaling on astrocyte EAAT-2 levels in the context of HAND. Supported by NIDA 5R01DA025566

Macrophage secretome validation in plasma of patients with HIV associated neurocognitive disorders

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Perivascular macrophages and microglia are the primary cells infected with HIV-1 in the brain. Upon infection, macrophages release proteins that may be related to neuronal degeneration and death. Our main hypothesis is that changes in the macrophage's secretome play an important role in the emergence of cognitive impairment in HIV-1 seropositive women. Macrophages were isolated from the peripheral blood of 9 HIV+ and 3 HIV- women, characterized for neurocognitive function, by adherence and differentiated in culture for 7 days. A quantitative proteomics approach using isobaric tag for relative and absolute quantitation (iTRAQ®) was used to detect differentially expressed proteins. When comparing the secretome of HIV+ cognitively impaired to normal cognition and asymptomatic HIV+ women, we found 14 proteins differentially expressed. Among these, S100A9, MMP-9, Corticosteroid binding globulin (CBG) and Gelsolin were candidates for validation by ELISA. Using plasma from 40 HIV-infected patients stratified by neurocognitive function, we found that CBG was decreased in patients with the worse cognitive function (MSKN=2) when compared to asymptomatic (p<0.05) or normal cognition (p<0.01) patients. S100A9 showed a tendency to decrease in asymptomatics when compared to normal cognition (p=0.0682) or cognitive impaired patients (p=0.0760). Differential expression of MMP-9 and Gelsolin was not observed by ELISA. S100A9 is pro-inflammatory; meanwhile, CBG is anti-inflammatory, therefore findings suggesting possible inflammation biomarkers for HIV-dementia development. Supported by R01-MH08316-01, 8G12-MD007600, SNRP-NINDS-1-U54NS43011, RISE G12RR03051, INBRE P20RR0164

Ligand Independent Activation Of Platelet Derived Growth Factor-beta Receptor During HIV-Tat And Cocaine Mediated Enhanced Proliferation Of Pulmonary Smooth Muscle Cells

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Neuroprotective effect of CCL5 via the G-protein coupled receptor 75 (GPR75) activation.

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The chemokine CCL5 affects the binding of the envelop protein gp120 to the co-receptor CCR5. CCL5 also prevents neuronal cell death mediated by the X4 gp120 and Tat, which have no affinity for CCR5. The mechanism of action of this chemokine remains to be fully characterized. Recent studies and preliminary data have shown that CCL5 activates a GPR75 which belongs to the Gq α family. This receptor is more abundant in the brain than in the immune organs. Moreover, CCL5 activates various pro-survival signaling molecules, including inositol triphosphate, phosphatidylinositol 3-kinase and its downstream targets, protein kinase B (Akt) and extracellular signal-regulated kinases (ERK1/2), in SH-SY5Y human neuroblastoma cells. These cells do not express CCR5, CCR3 and CCR1, receptors known to bind to CCL5. Moreover, CCL4, CCL7 and CCL3, other chemokines that bind to CCR5, CCR3 and CCR1, failed to activate these signaling molecules in SH-SY5Y cells. Akt and ERK1/2 phosphorylation were blocked by the Wortmannin and U73122, inhibitors of Akt and ERK1/2, respectively. At the same time these responses were insensitive to pertussis toxin, a Gi inhibitor, suggesting that CCL5 activates a GPCR coupled to Gq proteins. Therefore, GPR75 could explain the neuroprotective activity of CCL5 against gp120 and Tat. The discovery and characterization of compounds that prevent or limit the neurodegeneration that follows HIV infection of the brain is a great challenge for HIV research. Thus, the results provide new mechanistic insight which can be instrumental in addressing this challenge. Supported by NS 079172, NS 074916, DA 032282

Longitudinal changes in CSF metabolites as a prognostic marker for cognition in HIV-infected patients

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There is an unmet need for surrogate markers which can predict which HIV+ patients will develop neurocognitive disorders. Here, we analysed CSF samples from 98 HIV+ patients with differing neurocognitive states using 1H-NMR spectroscopy to identify metabolomic biomarkers for cognitive impairment. Two samples were collected from each patient from four neurocognitive states that were defined by temporal changes in cognitive status. These neurocognitive states were stably-normal, stably-abnormal, improving, and declining. It was possible to longitudinally separate each neurocognitive state using multivariate PLS regression modelling with q2 values = 0.61 (normal), 0.84 (abnormal), 0.47 (declining) and 0.52 (improving). The metabolites that underpinned these changes were distinct in each group, but were generally connected to amino acid, and energy metabolism. Using a classification and regression tree analysis on the NMR data and clinical variables we identified the following markers which were prognostic indicators for the declining patients: pyroglutamate, citrate, creatine, alanine, serotonin, low nadir CD4 count and a detectable plasma viral load (sensitivity 96%, specificity 80%). The following prognostic indicators were identified for the improving patients: glutamate, pyroglutamate, creatine, myoinositol, and β -glucose (sensitivity 91%, specificity 92%). These findings suggest that declines in cognitive function are accompanied by a variation in specific energy metabolites. These energy metabolites could be useful surrogate markers for HAND.

Soluble Neurotoxins from HIV-infected Macrophages cause Calpain-dependent Necdin Cleavage

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HIV-associated neurocognitive disorder (HAND) is attributed to the release of neurotoxic soluble factors from HIV-infected macrophages in the CNS. Features of HAND include aberrations in neuronal cell cycle proteins including E2F1. Prior work has shown that cytoplasmic levels of E2F1 increase in HAND, and this can activate calpain. Loss of E2F1 function attenuates neuronal death in models of HIV-induced neurotoxicity and E2F1 is cleaved to a stable fragment by calpain, but does not induce apoptosis through transcriptional activation. These findings suggest that E2F1 cleavage contributes to neuronal death in HAND, and that unique regulators of E2F1 in neurons may influence this process. Necdin is a protein involved in induction and maintenance of post-mitotic status of neurons. Necdin physically interacts with E2F1, and protects neurons against apoptosis during activity deprivation. Therefore necdin might be degraded via an E2F1-dependent mechanism in a model of HIV-induced neurotoxicity. Exposure of primary rat neuroglial cultures to supernatants from HIV-infected human macrophages resulted in degradation of necdin, which was dependent on NMDA receptor and calpain activation. Intriguingly, we found that necdin is upregulated in primary neuroglial cultures from mice lacking transcriptionally active E2F1. Finally, we found that necdin is cleaved by calpain to a smaller molecular weight in our in vitro model. These findings suggest molecular interplay between E2F1 and necdin in neurons that may be disrupted in HIV-induced neurodegeneration. Supported by NIH/R01NS041202 and T32ES019851-02

Transcriptomic analysis of HIV infected, cART, and METH treated macrophages: METH alters control of gene expression in treated infection

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Combination antiretroviral therapy (cART) is beneficial in stopping accumulation and spread of HIV throughout the body, yet does not clear virus from reservoirs such as macrophages. The use of methamphetamine (METH), is common among those infected with HIV, and complicates an already compromised environment. We hypothesized that HIV infection alters the macrophage transcriptional network resulting in macrophage functional alterations, some of which may be restored by treatment with cART but can be altered by treatment with METH. This systems biology study, investigates changes of the macrophage transcriptome during HIV infection, cART and METH treatment. Using RNAseq we determined the transcriptome profiles of each condition (MDMs from 5 donors $\pm$ HIV, cART, and/or METH) and differentially expressed genes were identified. We assessed protein-protein interaction

networks driving the transcriptome profile. Following the addition of cART treatment to the HIV infected MDMs, a large number of transcriptional regulators were no longer involved in the network. Interestingly most of these genes encoded proteins functioning in apoptosis. Surprisingly, when cART treated HIV infected MDMs were treated with METH, the network became more complex, revealing that METH use has a striking impact on HIV infected macrophages under cART treatment. These data suggest that use of METH can have profound effects not only on the gene expression patterns but the function of macrophages in cART treated, HIV infected individuals. The nature of such effects are now under investigation. Supported by R01 DA030962

Interleukin-1 beta released by gp120 mediates upregulation of neuronal ferritin heavy chain and may contribute to synaptic injury in HIV infection

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HIV-associated neurocognitive disorders are characterized by a number of pathophysiologies, including synaptodendritic injury. We propose that HIV proteins and inflammatory cytokines cause synaptic damage by increasing neuronal expression of ferritin heavy chain (FHC), a known negative regulator of the CXCL12/CXCR4 signaling axis. Our results suggest that TNF-alpha and IL-1 beta, as well as the HIV envelope protein gp120, upregulate FHC in neurons, albeit in different manners. Unlike the inflammatory cytokines, gp120 (X4- or R5-using) only caused significant increases in neuronal/glia bilaminar co-cultures, suggesting glia are necessary for elevations in FHC. In support of this, the presence of an IL-1 beta neutralizing antibody or receptor antagonist blocked gp120-mediated increases in neuronal FHC. Surprisingly, while gp120 could induce both IL-1 beta and TNF-alpha secretion from glial cells, a TNF-alpha neutralizing antibody did not prevent gp120-induced upregulation of FHC. In two in vivo models of HIV infection (gp120-ICV injected and HIV-Tg rats), we saw a significant reduction in cortical neuron dendritic spine density compared to age-matched controls. Preliminary experiments in HIV-Tg animals have also revealed elevations of FHC in whole brain lysates. Overall, these studies suggest that HIV infection and opiates may converge on neuronal FHC and lead to impairment of important homeostatic mechanisms, including dendritic spine stability. Supported by NIH DA15014 & DA32444

Alcohol accentuates simian immunodeficiency virus (SIV)-induced alterations in the skeletal muscle milieu contributing to impaired satellite cell myogenic differentiation

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Alcohol abuse and HIV infection independently result in systemic pro-oxidative and pro-inflammatory milieus. Previous studies in our lab demonstrated that chronic binge alcohol (CBA) administration during simian immunodeficiency virus (SIV)-infection promotes a pro-oxidative and pro-inflammatory milieu in skeletal muscle of SIV-infected male macaques. In addition, our studies have demonstrated that CBA accentuates muscle wasting in AIDS and impairs the ex vivo myogenic differentiation of satellite cells (SCs). We hypothesize that the SCs microenvironment affects the repair/regeneration of myofibers. We examined one of the critical pathways that has been demonstrated to negatively regulate SC activation and differentiation: transforming growth factor- β (TGF- β). TGF-B1 is secreted by active immune cells, platelets and SCs themselves. Latent TGF-B1 that is bound locally to the extracellular matrix can also be activated after injury. Using muscle tissue or SCs isolated from CBA or sucrose-fed SIV-infected rhesus macaques, we demonstrate that there is a significant increase in TGF-B1 expression in the muscle and decreased myogenic differentiation of SCs into myotubes in the CBA-SIV animals. There was also increased expression of Smad2 in the CBA-SIV SCs and increased Picrosirius red staining in muscle isolated from CBA-SIV macaques, supporting fibrosis in the muscle tissues. The results provide evidence of dysregulation of the skeletal muscle milieu due to chronic alcohol in SIV infection that impairs SC myogenic differentiation. Supported by NIH P60AA09803

Methamphetamine synergistically interacts with HIV-1 Vpr in the induction of IL-6 and IL-8 in astrocytes

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Both HIV-1 accessory protein Vpr and methamphetamine have been shown to play an important role in the development of neurocognitive impairments. However, it is not known if these two will have additive effects via production of proinflammatory cytokines. Furthermore, methamphetamine has been shown to accelerate the virus replication in in-vitro and in-vivo studies that would lead to increased production of viral proteins. To address this question, we treated SVGA astrocytes with various concentrations of methamphetamine for 3 days and transfected them with either mock or HIV-1 Vpr encoding plasmid. We observed methamphetamine (10 μ M, 100 μ M and

500µM) dose dependent synergistic induction of IL-6 by HIV-1 Vpr. However, induction of IL-8 showed synergy only at the lower doses of methamphetamine (10µM and 100µM). On the contrary no additive effect was observed in terms of CCL5 production. p38MAPK, PI3K/Akt, NF-κB, AP-1 and C/EBP pathways were examined using pharmacological antagonists and siRNAs to determine the signaling pathways for IL-6 and IL-8 synergy. Our results indicate that all these pathways are involved in synergistic interaction between methamphetamine and HIV-1 Vpr in astrocytes. This is first report showing evidence for synergy between HIV-1 Vpr and methamphetamine which will be expected to have significant implication in development of HIV-associated neurological disorders (HAND). Supported by NIH grants DA025528, DA025011 and AA020806 to Dr. Anil Kumar

HIV protease inhibitors promote neuronal Aβ42 production via ER stress-induced translational upregulation of BACE1

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The advent of antiretroviral therapy (ART) as the mainstay for HIV treatment has led to a reduction in the incidence of HIV-associated dementia. However, the prevalence of mild forms of HAND has persisted despite ART. In addition, HIV-associated neuropathology has evolved from a subacute encephalitis to a prolonged, neurodegenerative disease. While increased longevity of HIV(+) populations is thought to contribute to alterations in HAND pathology, other risk factors, such as peripheral toxicities of ART and potential central effects linked to accelerated aging, remain largely unexplored. To evaluate ART-mediated neuronal toxicity we treated rat neuroglial cultures with therapeutically relevant concentrations of reverse transcriptase inhibitors (RTI), and HIV protease inhibitors (PI). We found that PIs, but not RTIs, led to a significant loss of MAP2 indicating neuronal damage/death. Given the known role of PIs in activating ER stress, we explored the unfolded protein response in neurons as a potential pathway contributing to cell death. We found that PIs induce neuronal ER stress leading to PERK-dependent phosphorylation of eIF2α and enhanced translation of the β-secretase enzyme, BACE1. These effects were abrogated by PERK excision or overexpression of kinase-dead PERK mutants. Additionally, we observed PI-mediated amyloidogenesis in CHO cells expressing human APP, while ART administered to rats or SIV-infected macaques resulted in neuronal damage and ER stress in the CNS. Together, these results implicate PIs as potential mediators of neurodegeneration in HAND.

Alcohol induced up-regulation of purinergic receptor (P2X4) expression in microglia: A distinct role of MEK-ERK signaling pathway

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Purinergic receptors play a key role in regulating microglial function, and more significantly, purinergic receptors have been shown to be sensitive to alcohol-induced effects. We have previously shown that alcohol increases P2X4R expression altering microglia functions. Furthermore, pharmacological blockade with a selective P2X4R antagonist could reverse the action, suggesting that P2X4R may play a role in mediating alcohol-induced effects on microglia. P2X4R plays an important regulatory function in microglia. It is involved in a myriad of molecular signaling such as proliferation, activation of transcription factors, specifically through the MAPK pathway; and ATP signaling. Here, we investigate the intracellular signal transduction pathway that influences P2X4R expression in microglia in response to alcohol. We found alcohol (100mM) phosphorylated mitogen-activated protein kinase (MAPK kinase, MEK) and extracellular signal-regulated kinase (ERK) cascades. Inhibition with a MEK pharmacological inhibitor U0126 prior to alcohol treatment resulted in partial inhibition of MEK-ERK phosphorylation and was associated with a decrease in P2X4R expression. Studies are underway to explore the downstream signaling effects of MEK-ERK pathway and their role in transcription factor activation mediating the alcohol-induced P2X4R expression. Together, these results suggest that the MEK-ERK signaling cascade has a distinct role in the up-regulation of P2X4R expression in microglia.

Effects of chronic HIV-1 Tat exposure in the CNS: heightened vulnerability of males versus females to changes in cell numbers, synaptic integrity, and behavior

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HIV-associated damage to the CNS results in cognitive/motor deficits. Anti-retroviral therapies reduce the severity of symptoms, yet the proportion of patients affected has remained the same or increased. Although approximately half of HIV-infected patients worldwide are women, whether biological sex influences outcome of HIV infection has been controversial. We performed both behavioral and cellular/morphologic endpoints, using a transgenic mouse that inducibly expresses HIV-1 Tat in brain. After 3 mo of HIV-1 Tat exposure both sexes showed similarly reduced open field ambulation. Male Tat+ mice also showed reduced forelimb grip strength, and enhanced anxiety in a light-dark box assay. Tat+ males did not improve over 12 wk of repeated rotarod testing, indicating a motor memory deficit. Male mice also had more cellular deficits in striatum. Both had equally reduced oligodendroglial populations, and equivalent microglial increases. However, astrogliosis and microglial nitrosative stress were higher in males. Dendrites on medium spiny neurons in Tat+ male had fewer spines, and levels of excitatory and inhibitory pre-and post-synaptic proteins were disrupted. Our results predict sex as a determinant of HIV effects in brain. Increased behavioral deficits in males correlated with glial activation and synaptic damage, both of which are implicated in cognitive/motor impairments in patients. Tat produced by residually infected cells despite antiretroviral therapy may be an important determinant of synaptodendritic instability and behavioral deficits accompanying chronic infection. Supported by NIH/DA024461 & NS069216 (PEK)

Induction of miR-21 by cytokines and growth factors, a potential neuroprotective response to nervous system inflammation and injury

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Inflammation and injury in the brain are complex and highly regulated processes. MicroRNAs (miRNAs), which are known to be important for inflammation in many tissue types can regulate the expression of hundreds of proteins by translational inhibition mRNA targets. Our work aims to examine the expression and regulation of miRNA during neuroinflammation and elucidate their functional role in CNS injury and disease. Studies on HIV and SIV encephalitis in our lab revealed increased expression of miR-21 and miR-142 in the brain. Interestingly, miR-21 increases in several other CNS insults including stroke, traumatic brain injury (TBI), radiation, and epilepsy. This suggests that miR-21 is a common response to CNS injury and inflammation. We examined the effect of various cytokines and growth factors associated with CNS injury and inflammation on the expression of miR-21. We found that miR-21 is induced by IL-6, BDNF, and CNTF in primary human neurons. CNTF showed the strongest induction of miR-21 among the factors examined. Bioinformatic analysis of experimentally validated miR-21 targets revealed that several are known to promote neuronal death, suggesting that miR-21 may promote neuronal survival. Future studies will examine the potential neuroprotective role of miR-21 in CNS injury. Although CNTF upregulates miR-21, and its administration has shown benefits in animal models of CNS disease, clinical trials have failed due to negative side effects. Targeting downstream signaling events such as miR-21 or its targets may be an effective alternative therapeutic strategy. Supported by DHHS/NIH/NIDA/grant 5R01DA030962-03

Brain pericyte dysfunction in chronic inflammation caused by HIV-1 infection

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Pericytes of the central nervous system are uniquely positioned within the neurovascular unit to provide crucial support to blood brain barrier (BBB) formation, maintenance, and stability. Many neurologic conditions, such as HIV-1 associated neurocognitive disorder (HAND), are associated with BBB compromise due to chronic inflammatory responses. Little is known about altered pericyte function during HIV-1 infection and the subsequent contribution to HAND. In this study, we examined functional changes of primary human brain pericytes exposed to HIV-1 and the inflammatory cytokines, TNF α and IL-1 β . First, we established culture conditions for a quiescent, non-proliferating phenotype of human brain pericytes by diminution of growth factors in the media without effect on their viability. Next, we assessed expression of pericyte markers [α 1 integrin, α -SMA, and PDGF-R β] and CX-43 (a gap junction marker) by western blot, qPCR, and FACS. We found down regulation of α 1 integrin, α -SMA, PDGF-R β and CX-43 in pericytes exposed to TNF α and IL-1 β . Secreted factors promoting BBB formation [Ang-1 and TGF- β 1] analyzed by ELISA showed significant decrease after exposure of pericytes to virus, TNF α or IL-1 β . Basement membrane components (fibronectin, nidogen) assessed by qPCR were reduced in pericytes treated with cytokines or HIV-1. TNF α and IL-1 β enhanced expression of adhesion molecules (ICAM, VCAM) in pericytes paralleling increased monocyte adhesion to pericytes. These data suggest an essential role of pericytes in BBB regulation during HIV-1 infection.

Region-Specific Contribution of the Ventral Tegmental Area to Heroin-Induced Conditioned Immunomodulation

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Previous studies in our laboratory have shown that the immunosuppressive effects of heroin can be conditioned to environmental stimuli. Recently, we have demonstrated that these conditioned responses are mediated via a circuit that exists between the nucleus accumbens (NAc) and basolateral amygdala (BLA). Furthermore, stimulation of D1 dopamine (DA) receptors within these brain regions is necessary to produce heroin-induced conditioned immune suppression. The current study investigates the role of the ventral tegmental area (VTA), the primary source of DA signaling to the NAc and BLA, on heroin-induced conditioned immunomodulation. The conditioning procedure consisted of repeated pairing of heroin (1 mg/kg) administration with placement into a distinctive environment. On test day, animals received bilateral intra-VTA microinfusions of a mixture of the GABA agonists baclofen/muscimol (0.03 nmol/0.3 nmol) to temporarily inactivate the rostral or caudal region of the VTA prior to re-exposure to the conditioned stimulus (CS). Following removal from the CS, animals received an injection of lipopolysaccharide (LPS) to induce an immune response. Spleen tissue, brains and blood were collected six hours following LPS injection. Real-time RT-PCR was conducted to measure cytokine mRNA expression and nitric oxide production. ELISAs were also conducted to measure cytokine protein levels. Results indicate that inactivation of the rostral, but not caudal, VTA inhibited the immunosuppressive effects of the CS, suggesting a role for the anterior VTA in heroin-conditioned immunomodulation. Supported by grant DA25667 from the National Institute on Drug Abuse

Layer-by-Layer (LbL) Assembly of Anti HIV Drug for Sustained Release to Brain Using Magnetic Nanoparticle

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The advent of highly active antiretroviral (ARV) drugs has led to prominent reduction in plasma viral load, opportunistic infection and mortality from AIDS. Unfortunately most of these drugs have poor transport properties, relatively short half-life, low bioavailability, poor CNS penetration, retention with undesirable side effects. Further non adherence to medication and emergence of drug resistance in HIV infected patients lead to loss of therapeutic efficacy. Henceforth there is a need to develop novel drug delivery systems to overcome these challenges. Layer-by-Layer (LbL) electrostatic self assembly technique can be utilized to improve the management of drug therapy in HIV patients by delivering the drug at a controlled rate. We have chosen Tenofovir as a model drug and magnetic nanoparticle as drug carriers for the current study. Our result showed that application of one bilayer (1BL) of Dextran sulfate sodium (Mol wt >500000) leads to more than 110% drug encapsulation and prolong the drug release by 12 times (2 days) compared to drug loaded non-coated Iron oxide nanoparticles (Fe₃O₄) (4 hr), while two bilayers (2BL) leads to 30 times (5 days) prolongation of drug release with zero order kinetics. Further studies are underway to test the sustainability of the formulation using Blood Brain Barrier model and animal studies. In conclusion we can say that by controlling LbL assembly parameters (thickness, deposition time, polyelectrolyte coating and pH), we can substantially achieve our goal of sustained release of drugs for prolonged period (one week or more). Supported by NIDA, NIMH

Modeling HIV induced disruption of blood-brain barrier function in mice

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Widespread use of combined antiretroviral therapy (cART) has made a significant impact on the course of infection, dramatically extending the average lifespan of HIV infected individuals. However, as these individuals are living longer, many HIV-associated chronic inflammatory illnesses are becoming prevalent. HIV-associated neuroinflammation is believed to be a major catalyst in the development of HIV-Associated Neurocognitive Disorders (HAND), which are estimated to persist in approximately 50% of infected individuals regardless of cART. We have previously demonstrated that the inflammatory mediator soluble CD40 ligand (sCD40L) is elevated in both the plasma and cerebrospinal fluid of cognitively impaired infected individuals as compared to their non-impaired infected counterparts, presumably via platelet-dependent mechanisms. We have recently demonstrated an increasing role for this inflammatory mediator in HAND pathogenesis, thereby identifying this molecule as a potential therapeutic target for the management of HAND. To recapitulate the inflammatory aspects of HIV-1 infection, we recently infected wildtype mice with ecotropic HIV (EcoHIV), a chimeric virion derived from murine leukemia virus. Two months post infection, these infected mice demonstrated marked increase in platelet

activation markers and disruption of blood-brain barrier (BBB) permeability, distinguishing features of HAND. Collectively, our data underscores potential of this novel animal model in studying inflammatory disorders associated with HIV-1 infection, and warrants further investigations. Supported by NINDS/NIH

Central infusion of interleukin-1 receptor antagonist prevents the development of stress-enhanced fear learning

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Post-traumatic stress disorder (PTSD) is a pathological condition in which a severe trauma produces debilitating psychological and physiological consequences. Recently, morphine treatment after a trauma has been associated with reduced rates of PTSD. Similarly, we found that morphine administration prevented the development of stress-enhanced fear learning (SEFL), an animal model of the disorder. Here, we test the hypothesis that neural cytokines are a key mechanism of morphine's action in preventing SEFL. In experiment 1, rats were exposed to the severe stressor of SEFL, and were sacrificed immediately, 6, 24, 48, or 72h later. Brain sections were stained with primary antibodies against interleukin-1 β (IL-1 β). Total plasma corticosterone (CORT) was measured by radioimmunoassay. In experiment 2, rats were implanted with intracerebroventricular cannulae and subjected to the SEFL paradigm. At 24 and 48 hours after the severe stressor, animals were administered either saline vehicle or 10 μ g of IL-1 receptor antagonist (IL-1Ra). In experiment 1, IL-1 β was enhanced in the dorsal hippocampus at 6 through 72h. As expected, plasma CORT was significantly elevated immediately after the stressor and returned to baseline at 6h. Interestingly, CORT was re-elevated at 48 and 72h after the stressor. In experiment 2, IL-1Ra administration significantly attenuated fear learning to a subsequent single shock, i.e. prevented stress-enhanced fear learning. These results support the hypothesis that IL-1 β may be a target of morphine's action. Supported by T32 DA07244

Using plasma microRNAs as biomarkers for cognitive impairment in HIV patients

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Neurocognitive impairment and depression adversely impact HIV treatment adherence and identification of early markers of neurocognitive impairment could lead to interventions that improve psychosocial functioning and slow disease progression through improved treatment adherence. Evidence has accumulated for the role of miRNAs in neurocognitive disorders based on analysis of cerebrospinal fluid (CSF) and brain tissue, but these are not practical approaches for predicting/identifying neurocognitive disorders in the clinic. We have developed an accurate and sensitive protocol to profile miRNAs in body fluids and we hypothesize that plasma miRNAs may associate with subclinical neurocognitive impairment (CI), HIV treatment adherence, and HIV disease outcomes. 51 HIV+ patients with unknown neurocognitive status have thus far been recruited from the LSUHSC HIV Outpatient Clinic for this prospective cohort study. During routine clinic visits, validated assessments are performed for CI and depression, and whole blood is collected for miRNA profiling. 27 of 51 patients (53%) demonstrated CI. Moreover, 34 of 51 patients (67%) met predetermined criteria for depression. After controlling for HIV disease status and other factors associated with CI, miRNA signatures associated with CI and depression were identified. Ongoing analyses will determine whether miRNA profiling offers a practical approach for identifying HIV+ patients with CI which are potentially amenable to interventions which, along with antiretroviral therapy, may improve HIV disease outcomes for these high-risk patients. Supported by 1 U54 GM104940 from the National Institute of General Medical Sciences of the National Institutes of Health

Electrochemical Immunosensor for the Detection of Cortisol

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Psychological stress caused by everyday life style contributes to health disparities experienced by individuals. Cortisol level has been shown to increase on human immunodeficiency virus (HIV) infection and in recreational drug users. Here in the present work, a simple, low-cost, label-free, electrochemical immunosensing platform is developed for sensitive and selective detection of saliva cortisol. Electrochemical immunosensing is utilized for the detection of cortisol using Anti-Cortisol antibodies (Anti-Cab) covalently immobilized on self-assembled monolayer (SAM) of dithiobis(succinimidylpropionate) (DTSP) modified microfabricated interdigitated microelectrodes (IDEs). The non-binding sites of immunosensor surface were blocked using ethyleneamine (EA). Electrochemical response studies of EA/Anti-Cab/DTSP-SAM/IDEs as function of cortisol concentration exhibited a detection range from 10 pg/mL to 100 ng/mL, a detection limit of 10 pg/mL, and a sensitivity of 6 μ A/(pg/mL) with the regression

coefficient of 0.99. The obtained sensing parameters were in physiological range and fabricated immunosensors were also tested successfully on clinical sample of saliva collected at different time zones of two specimen. Serum samples obtained from the cocaine using and non-using HIV patients will be studied for cortisol level using the newly invented electrochemical immunosensor and data will be presented. Supported by NIH, NSF

Cannabinoids modulate cytokine profiles within HIV-1-infected individuals in the DREXELMED HIV/AIDS Genetic Analysis Cohort

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This study was performed to evaluate the relationship between the use of cannabinoids and impact on cytokine modulation and HIV-1 disease severity in HIV-1-infected patients enrolled in the DREXELMED HIV/AIDS Genetic Analysis Cohort. Within the DREXELMED HIV/AIDS Genetic Analysis Cohort, patients are assessed approximately every 6 months for HIV-1 clinical markers and history of illicit drug, alcohol, tobacco and medication use. The Luminex human cytokine 30-plex panel was used for cytokine quantitation. Analysis was performed using a newly developed biostatistical model. Substance abuse was found to be common within the cohort. Utilizing the drug screen information from each visit, subjects in the cohort were categorized as preferential nonusers, cannabinoid users, and multidrug users. Clinical parameters such as viral load, CD4+ and CD8+ T-cell counts as well as CD4:CD8 ratio were not impacted by increased cannabinoid use. Among the 30 cytokines investigated, IL-1 α , IL2, IL-1A, and IL-5 were positively associated with cannabinoid use and the levels of MCP-1 negatively associated. Due to the connection between MCP-1 and neurocognitive impairment, and the fact that MCP-1 was downregulated in cannabinoid users, HIVD scores within the cohort were associated with cannabinoid use and the highest level of cannabinoid use was found to be neuroprotective in nature. In conclusion, cannabinoids do not appear to facilitate HIV-1 disease progression based on these assessments and may have neuroprotective properties. Supported by This work is supported by NIH/NINDS R01 NS32092, NIDA R01 DA19807, NIMH P30 MH092177, and NIMH T32 MH079785.

Morphine Disrupts Leukocyte Extravasation through alterations in cell motility

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Morphine inhibits immune cell recruitment resulting in increased bacterial dissemination, reduced bacterial clearance, wound healing and angiogenesis. Additional studies revealed that when compared to placebo, morphine administration in mice led to greater circulating levels of leukocytes; suggesting that the underlying mechanisms associated with morphine inhibition in recruitment is due to defects in leukocyte extravasation. Leukocytes undergo changes in morphology through F-Actin polymerization. Morphine modulates F actin reducing bacteria induced phagocytosis. We hypothesized that morphine alters signaling involving F-actin polymerization leading to defects in leukocyte extravasation. To test this, we used a PCR array to identify genes involved in cell shape and motility, using mouse leukocytes. Promising targets were validated in human leukocytes treated with an inflammatory stimulus, LPS and hypoxia using real-time PCR. Morphine (1.0 μ M) significantly down-regulated the mRNA expression of 37 and up-regulated 4 genes (*p<0.05) 2 fold or greater out of 86 genes analyzed. Morphine decreased genes associated with leukocyte chemotaxis, cell-cell adhesion, membrane blebs and invasive projections. In human cells, the inflammatory stimulus, and hypoxia differentially regulated genes. While the presence of message RNA does not indicate the availability of or direct translation of protein, these results suggest that morphine alters the ability of leukocytes to mediate and maintain, cell-cell adhesion and invasive projections for transmigration. Supported by NIH NIDA

Interleukin-1 β in the dorsal hippocampus as a novel mediator of heroin conditioned immunosuppression

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Heroin suppresses the immune system, and exposure to environments associated with heroin use can also produce immunosuppression. The dorsal hippocampus (DH) is necessary for this learned effect. Pro-inflammatory cytokine, IL-1 β , is linked to learning and memory and is expressed in the DH. Present studies investigated the role of DH IL-1 β in heroin conditioned immunosuppression and food conditioned place preference (CPP). Rats were conditioned to associate heroin with a context. They were then microinjected with IL-1 β or missense control siRNA in the DH prior to re-exposure to the heroin-paired context. Immediately afterward, the immune system was challenged and splenic iNOS and plasma nitrate/nitrite levels were assessed. In another cohort, CPP was established for a food-paired context and rats received microinjections before CPP re-testing. IL-1 β siRNA prevented heroin-paired context induced suppression of the normal splenic induction of iNOS (p0.05), indicating that IL-1 β suppression does not disrupt memory in general. These studies reveal what could be a specific mechanism for IL-1 β in heroin or conditioned immune responses that lead to unique intercellular signaling. Understanding IL-1 β 's precise action will critically characterize how heroin engages memory and immune systems and possibly provide therapeutic targets to help restore immune function in drug users. Supported by NIDA Grants DA 25667 and T32 DA 07244

HIV protein Tat induces oligodendrocyte damage via Kv1.3

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HIV-associated dementia is a subcortical dementia involving axonal demyelination or/and damage. Axonal myelination, wrapped by oligodendrocytes (OLs), is highly regulated by neuronal and astrocytic signals and maintenance of myelin sheaths is a very complex process. It has been shown that OLs are sensitive to viral proteins such as Tat and myelin damage is apparently associated with a decrease in numbers of OLs. It has also been shown that enhancement of outward K⁺ current results in neuronal apoptosis. We hypothesize that HIV protein Tat induces OLs injury by enhancing outward voltage-gated K⁺ channel 1.3 (Kv1.3) current, leading to OLs apoptosis and resultant demyelination. To test this hypothesis, we studied involvement of Kv1.3 (a Kv channel expressed in OL lineage cells) in Tat-induced OLs injury in vitro and ex vivo. Incubation of primary rat OL cultures with Tat increased levels of Kv1.3 expression, enhanced outward K⁺ currents induced by voltage steps, decreased expression of myelin basic protein (MBP), induced OL apoptosis as revealed by TUNEL staining and impaired axonal myelination in cultured brain slices as revealed by Luxol Fast Blue staining. These Tat-associated changes can be attenuated or blocked by PAP, a specific Kv1.3 blocker. Taken together, our results indicate Tat induces axonal damage through Kv1.3-associated myelin sheath impairment, suggesting Kv1.3 could be a potential target for the development of therapeutic strategies for HIV-associated white matter injury. Supported by NIH grant R01 NS 077873

PI3K/Akt and p38 MAPK pathways and transcription factors NF-kB, CEBP, and AP-1 are involved in HIV-1 Nef-mediated Increase of CCL5 in Astrocytes

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HIV-associated neurocognitive disorders (HAND) are a persistent problem in HIV-1 infected individuals. The production of pro-inflammatory cytokines by astrocytes/microglia exposed to viral proteins including Nef is thought to be one of the primary mechanisms leading to HAND. In the present study we examined the effect of Nef on CCL5 induction in astrocytes. CCL5 mRNA and protein levels are significantly induced in a time-dependent manner in Nef-transfected SVGA astrocytes. The peak level of CCL-5 mRNA was 24.6 $\pm$ 1.6 fold at 3 h post-transfection and the increase in CCL5 was visualized using fluorescent microscopy. To determine the mechanisms responsible for the increased CCL5 caused by Nef, pharmacological inhibitors and siRNAs were employed to determine the roles of the PI3K/Akt/NF-kB and p38 MAPK/CEBP/AP-1 signaling pathways. The antagonists of NF-kB (Bay11-7082), PI3K (LY294002), and p38 (SB203580) significantly reduced the mRNA and protein expression levels of CCL5 induced by Nef transfection. Furthermore, specific siRNAs against Akt 2 and 3, p38 α and δ , as well as different isoforms of NF-kB, CEBP, and AP-1 also significantly decreased Nef-mediated CCL5 expression at both mRNA and protein levels. This suggests that the PI3K/Akt and p38 MAPK pathways, along with the downstream transcription factors NF-kB, CEBP, and AP-1, are involved in Nef-induced pro-inflammatory cytokine production by astrocytes. This induction may be responsible for Nef-mediated toxicity in the central nervous system. Supported by National Institute on Drug Abuse grants DA025528 and DA025011 to AK

Intracerebroventricular infusion of IL-1 receptor antagonist decreases enteropathy and lung inflammation caused by HIV-1 Nef expression in the rat hippocampus

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Gastrointestinal (GI) and lung pathologies still represent a major burden to HIV patients even when combination antiretroviral therapies (cART) successfully control viral replication. Despite the use of cART, HIV replication and protein expression still occur in the brain. Our previous work has shown that Nef expression in the rat hippocampus increases blood-brain-barrier permeability, and induces enteropathy and lung inflammation, but did not address the role of brain inflammation. Here we tested our rat model for changes in the GI and lungs in response to the central administration of an anti-inflammatory drug. IL1 receptor antagonist (IL1ra) or saline (control) were infused for two days into the right ventricle of 30-day-old rats. On the second day, astrocytes expressing Nef were infused into the right hippocampus. Two days after recovery rats were euthanized to harvest the ileum and lungs. To assess alterations in GI secretion, changes in ion transport in response to acetylcholine were measured. H&E staining was performed on lung and ileal tissue to assess changes in morphology and inflammation. IL1ra rats show decreased secretion in ileal and colon tissue when compared to the saline rats. H&E staining showed that IL1ra rats have decreased inflammation in the ileum and the lungs showed decreased neutrophil and eosinophil infiltration and reduced interstitium thickness when compared to our control group. These findings suggest that inflammation in the brain induced by Nef expression may play an important role in the GI and lung pathology observed in HIV patients. Supported by R25GM082406, RR003050, MD007579, GM008239

Beta-catenin positively regulates key proteins in glutamate cycling in vivo

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Neurological disorders including HIV-associated neurocognitive disorders (HAND) have been linked to abnormal excitatory neurotransmission. Astrocytes play a key role in excitatory signaling as they both clear glutamate from the synaptic cleft and house enzymes responsible for glutamate conversion to glutamine. However, mechanisms responsible for the regulation of glutamate cycling including the excitatory amino acid transporter 2 (EAAT2) or GLT-1 in rodents and glutamine synthetase (GS) which catalyzes glutamate, remains largely undefined. We show that beta-catenin, a transcriptional co-activator and the central mediator of Wnt/beta-catenin signaling pathway, regulates both EAAT2 and GS expression in astrocytes in vitro. We also assessed whether beta-catenin regulates these two proteins in vivo by injecting control or vivo-morpholinos (500nM) to knockdown beta-catenin in the prefrontal cortex (AP+2.0, ML+/- 0.3, DV -1.0) of C57 BL/6 male mice by microinjection. Morpholinos, which block translation initiation of their gene target, were injected at day 0 and day 3. At day six, brain was collected and processed. We demonstrate that knockdown of beta-catenin resulted in a significant reduction in GLT-1 and GS protein expression by 99 and 93 percent respectively. These studies confirm that beta-catenin regulates key proteins responsible for excitatory glutamate neurotransmission in vivo and reveal the therapeutic potential of Wnt/beta-catenin modulation in treating diseases with abnormal glutamatergic neurotransmission and excitotoxicity. Supported by R01NS060632 to LAH

Astrocytic Nef increases endogenous TGF β secretion and induces SMAD in co-cultured neurons

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As the life expectancy of HIV-1 patients under treatment improves, mild forms of neuropathology have also increased. Controlling the replication of the virus does not prevent the production of early viral proteins such as Nef from latently infected cells including astrocytes. HIV infection and expression of Nef in astrocytes are each associated with cognitive impairments in HIV-1 patients. We have reported on a model of astrocytic Nef neurotoxicity leading to spatial learning impairment. In this same model, administration of an inhibitor of TGF β -1 signaling abrogates the learning deficit. In this work we studied the expression and target of TGF β -1 induced by Nef. We cultured SVGA astrocytes transfected with Nef to test for expression of TGF β -1 and activation of its signaling through SMAD. We also set up co-cultures with NT2 neurons exposed to these astrocytes. We found that Nef stimulated a rapid increase in TGF β -1 mRNA and secreted protein in astrocytes, assessed by real time RT-PCR and ELISA, respectively. We observed no evidence of SMAD activity induction by Nef in astrocytes. However, the exposed neurons showed activation of SMAD signaling. Based on the previous findings of our animal model, further characterization of roles of TGF β as well as the SMAD pathway in Nef neurotoxicity might be essential for elucidating adjunct therapy for HIV-1 patients suffering from cognitive disorders. Supported by GM106970, R25GM082406, and HD007579

Effects of HIV-1 Tat and morphine exposure on tight junction RNA and protein expression in a blood-brain barrier model.

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Approximately one-third of human immunodeficiency virus type 1 (HIV-1) cases resulting in acquired immunodeficiency syndrome (AIDS) in the United States have been linked to injection drug use within this population. Opioid use explicitly exacerbates disease progression, enhancing viral replication and peripheral viral load, as well as increasing incidence and severity of neurocognitive impairment, as compared to non-users. An altered blood-brain barrier (BBB) is directly involved in the pathology associated with the processes that ultimately result in HIV-1-associated neurocognitive disorders (HAND). HIV-1 proteins, as well as certain drugs of abuse, have been implicated in the compromise of the BBB. Previous studies have suggested that exposure to both HIV-1 Tat protein, as well as mu-opioids, alters BBB permeability, resulting in increased cellular transmigration, as well as overall barrier leakiness. In this study, a human brain microvascular endothelial cell line, hCMEC/D3, was utilized to establish an in vitro model of the BBB to investigate the effects of Tat and/or morphine exposure on the tight junction proteins (TJPs) of the BBB. Changes in mRNA transcripts of TJPs were observed throughout the course of morphine exposure. At the protein level, TJP localization was analyzed following cell fractionation and western immunoblot analysis. Overall, these studies demonstrated that exposure to Tat and/or morphine induced changes in TJP expression patterns at both the mRNA and protein level. Supported by NIH/NINDS R01 NS32092, NIDA R01 DA19807, NIMH P30 MH092177, NIMH T32 MH079785

TRAUMATIC BRAIN INJURY-INDUCED NEUROINFLAMMATION IS CORRELATED WITH INCREASED ALCOHOL DRINKING IN RATS

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Traumatic brain injury (TBI) is characterized by a mechanical insult followed by a neuroinflammatory response. Clinical data shows that alcohol use pre-TBI increases risk for alcohol abuse post-injury. The underlying mechanisms of TBI-induced increases in alcohol drinking are not known. This study used a rodent model of TBI induced by lateral fluid percussion (LFP) to examine the correlation between TBI-induced neuroinflammation and post-TBI increases in alcohol drinking. Adult male Wistar rats were trained to self-administer alcohol vs. water on fixed-ratio 1 (FR1) schedules in a two-lever contingency during limited-access sessions over four weeks prior to randomization to Craniotomy + TBI, Craniotomy alone, and Naive groups. TBI was produced by LFP (+2 ATM; 25 ms). Post-TBI drinking was measured and compared to baseline levels and non-TBI controls. After sacrifice, neuroinflammatory markers for microglia activation and astrocyte activation were measured via immunohistochemistry (IHC). Our results show that pre-injury alcohol preference predicted alcohol drinking post-TBI. Injury severity (mild 2ATM pressure) was also positively correlated with increased post-TBI alcohol drinking. IHC showed diffuse activation of astrocytes and microglia at the site of injury. These results show that post-TBI escalated alcohol drinking in rodents mirrors the trends seen in the human literature and is associated with neuroinflammatory changes. The contribution of neuroinflammation to increased alcohol drinking post-TBI is currently under investigation. Supported by LSUHSC Alcohol and Drug Abuse Center and T-32 NIAAA-007577

High CSF Hypocretin-1 Levels Are Associated With High Fasting Blood Sugar

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BACKGROUND: The hypocretin system, also known as the orexin system, regulates the sleep/wake cycle, feeding behavior, and regulation of glucose homeostasis. It is known that HIV infected individuals have a higher prevalence of insulin resistance when compared with the general population. Thus, the objective of our study was to investigate whether CSF hypocretin-1 (hcrt-1) levels correlated with fasting blood sugar levels in an HIV-seropositive Hispanic women cohort. **METHODS:** In a retrospective study, we determined CSF hcrt-1 levels from 25 HIV-seropositive women without a history of drug abuse evaluated for metabolic syndrome. Subjects were divided into normal fasting blood sugar levels (70-100 mg/dL) and high fasting blood sugar levels (higher than 100mg/dL). CSF hcrt-1 levels were determined using the fluorescent immunoassay kit (Phoenix Pharmaceuticals),

with an intra- and inter-assay validity of 10 and 15 percent respectively. The association between normalized CSF hcr1 and fasting blood sugar levels was tested using non-parametric statistics. Statistical significance was determined at p less than 0.05. No differences in age, education, viral load, CD4, hepatitis C virus, BMI, and BDI were observed. RESULTS: There is a positive correlation between CSF hcr1 levels and higher fasting blood sugar ($r=0.430$, $p=0.032$) in HIV-seropositive women. No correlation was found between CSF hcr1 levels and BMI or waist circumference. CONCLUSION: Our findings suggest that the hypocretin system may be involved in regulation of glucose homeostasis in HIV-seropositive women. Supported by R21MH095524; R25MH080661; R25MD007607; U54MD008149; U54NS043011

Traumatic brain injury (TBI) in adolescent mice enhances cocaine-induced place preference

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Clinical evidence indicates a connection between traumatic brain Injury (TBI) and addictive tendencies; however, very few pre-clinical studies have been performed to understand the effect that brain injury may have on drug addiction. Therefore, we designed a pilot study to test the hypothesis that TBI exacerbates the reinforcing properties of cocaine in a biased, conditioned place preference (CPP) assay. Adolescent, six-week old C57BL/6 mice underwent craniotomy surgery, after which the mice sustained a single, moderate TBI (speed: 4.5m/s, depth of impact: 2.0mm, dwell time: 0.5s) to the right parietal somatosensory cortex using an electromagnetically driven piston (diameter: 2.0 mm). One-week post-TBI, an activity-monitoring assay was used to assess locomotor deficits arising as a result of surgical/impact procedures. Impacted animals showed no locomotor deficits when compared to adolescent control subjects. CPP pre-testing occurred 2 weeks post-TBI, followed by six days of non-contingent cocaine administration (10mg/kg) through intraperitoneal injection. The place preference shift in the drug-paired environment was significantly enhanced in all treatment groups receiving cocaine as compared to saline controls. Furthermore, mice sustaining a moderate TBI during adolescence exhibited a significant increase in cocaine-induced place preference as compared to uninjured controls receiving cocaine. These results suggest that adolescent mice sustaining a single, moderate TBI event may be increasingly susceptible to the reinforcing properties of cocaine. Supported by NIH T32 DA007237 (SFM) and NIH R01 NS086570 (SHR)

Tyrosine 88 and Lysine 92 of human dopamine transporter play a critical role in Tat-induced inhibition of dopamine transporter function

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HIV-1 Tat protein disrupts the dopamine (DA) neurotransmission by inhibiting DA transporter (DAT) function, leading to increased neurocognitive impairment in HIV-1 infected individuals. Through computational modeling and simulations, three functional residues in human DAT (hDAT) were predicted as potential recognition binding sites for Tat. We have demonstrated that mutation of tyrosine470 (Y470H) of hDAT attenuates Tat-induced inhibition of DA uptake by changing the transporter conformational transitions (Midde et al., 2013). The present study investigated the functional influences of mutations of tyrosine88 (Y88F) and lysine92 (K92M), two other predicted residues for Tat binding to hDAT, in Tat-induced inhibitory effects on DA transport. Compared to wild type hDAT, K92M but not Y88F reduced V_{max} without changes in the K_m . Both Y88F and K92M did not alter IC_{50} values for DA inhibition of DA uptake but increased DA uptake potency for cocaine and GBR12909, indicating that these residues do not overlap with the binding sites in hDAT for DA transport but are critical for these inhibitors. Y88F and K92M attenuated Tat-induced inhibition of DA transport observed in wild type hDAT. In contrast to the complete attenuation of zinc-induced increased WIN35,428 binding by Y470H, Y88F and K92M partially attenuated this effect of zinc. Thus, these results demonstrate Tyr88 and Lys92 along with Tyr470 as functional recognition residues in hDAT for Tat-induced inhibition of DA transport and provide mechanistic insights into identifying targets on the DAT for Tat binding. Supported by R01DA035714

Protection from Sustained Attention Deficits in the HIV-1 Transgenic Rat with the Phytoestrogen Metabolite S-Equol

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Deficits in executive function are a distinguishing feature of HIV-1-associated neurocognitive disorders (HAND) in the combination antiretroviral therapy (cART) era, progressing more rapidly during HIV-1 disease progression than those of other cognitive domains. We have demonstrated prominent temporal processing deficits in the HIV-1 Transgenic (Tg) rat with assessments of sustained attention, a component of executive function, which were ameliorated with the phytoestrogen metabolite S-equol, administered at 6-8 months of age. In the present experiment, performance on the sustained attention task was assessed while the animals were administered 0.2 mg of S-equol or sucrose for 60 days, beginning at 2-3 months of age. Rats were trained to discriminate light signals (100, 500, or 1000 msec duration) from non-signals (no illumination). The HIV-1 Tg animals that received S-equol acquired the task significantly more rapidly than the other groups. Long-term effects of S-equol were also observed; one month after the treatment ended, the HIV-1 Tg animals that received S-equol exhibited a high level of accuracy across all signal durations, whereas the control animals that received S-equol failed to reliably detect the 100 msec signal throughout the entire testing period. Thus, the present results suggest that S-equol can effectively protect against the neurocognitive impairment of HAND when administered early in the progression of HIV-1, and may continue to have beneficial long-term effects. Supported by DA013137, HD043680, DA031604

Human macrophage cell lines exhibit different methamphetamine-mediated induction patterns

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Methamphetamine (METH) abuse produces an array of health problems and neurological complications in humans. It induces behaviors that favor the exposure to infections such as Human Immunodeficiency Virus (HIV). In addition, METH is able to upregulate CCR5 on the surface of myelomonocytic HIV target cells, increasing the probability of infection and the vulnerability to overall inflammatory pathologies. In the brain, in particular, HIV-induced pathogenesis and viral load are aggravated by METH. The effects of METH are partially mediated by its interaction with macrophages and microglia, either directly through sigma1R and TAAR1, or indirectly through the induction of dopamine, which interacts with various dopamine receptors (DRs) on the surface of innate immune cells. These interactions result in the activation of transcription factors that trigger a gene expression program responsible for the macrophage/microglia-dependent immune response. We studied the differences of receptor expression and transcription factors in two macrophage cell lines, THP1 and U937, which are the most widely used models for investigating biological functions of differentiated macrophages. THP-1 cells are less mature cells of blood leukemic origin, while U937 cells are of histiocytic lymphoma origin arrested in a more advanced stage of differentiation (promonocyte/ monocyte). These cells may represent subsets of macrophages at different states of maturation and differentiation, with different functional outcomes upon METH treatment. Here we show that mRNA levels of a subset of METH sensitive receptors are differentially expressed in these two human macrophage cell lines, both at baseline levels and upon treatment with METH. Furthermore, we show that protein levels and kinetics of transcription factors and CCR5 differ between METH-treated THP1 and U937 cells. Together, our data begin to characterize differences in the expression of METH sensitive receptors in distinct human macrophage cell lines, and microglia, which may account for variances in inflammatory response patterns induced by METH treatment across models, or in vivo between brain and peripheral organs. This work is funded by NIDA R01 DA036164.

Differential expression of Ferritin Heavy Chain in rat cortical cells in response to morphine stimulation of mu-opioid receptor

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Ferritin Heavy Chain (FHC), a subunit of the ubiquitously expressed iron storage complex Ferritin, has recently been shown to be upregulated in the central nervous system (CNS) in response to HIV/SIV infection and illicit drug abuse. Previous research has suggested that an unexpected consequence of increased CNS FHC lies in its ability to inhibit homeostatic signaling of the chemokine receptor CXCR4, resulting in decreased dendritic spine density in cortical neurons. Interestingly, initial studies indicate that these changes correlate with disease progression and behavioral symptoms observed in HIV associated neurocognitive disorders (HAND). Furthermore, chronic morphine treatment (both in vitro and in vivo) caused an upregulation of FHC in cortical neurons, which corresponded with decreased levels of activated CXCR4, and decreases in the activation of downstream signals (i.e. ERK1/2 and Akt). New immunofluorescence and fractionation data reveal that FHC upregulation occurs primarily in the cytoplasm and neuronal processes, with little to no change of nuclear expression. This is spatially consistent with neuronal CXCR4 expression, suggesting more frequent interactions of these proteins, and consequent inhibition of signaling. Additionally, morphine does not alter FHC in astroglial cultures, and there are likely no sex related differences in this regard. Investigating other CNS cell types in addition to neuronal FHC upregulation may provide clues about the effects of drug abuse in HAND progression, and other neurocognitive disorders. Supported by DA032444, DA015014

Chronic nicotine treatment rescues spatial and contextual memory deficit in HIV-1 transgenic rats

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The prevalence of tobacco smoking among HIV-infected patients is high; however, no direct causal benefits of nicotine over the HIV-associated cognitive impairment has been identified. HIV-1 transgenic (HIV-1Tg) rats show similar neuropathological and behavioral features to those of HIV positive patients, including altered dopamine and cholinergic signaling, and memory impairment. The present study was designed to investigate the effects of chronic treatment with nicotine on cognitive impairment in adult male HIV-1Tg rats. To determine the effects of the chronic nicotine treatment on spatial and contextual memory, HIV-1Tg and F344 control rats were given a single daily injection of either nicotine (0.4 mg/kg nicotine, base, s.c) or saline for 14 days. Rats were first tested for spatial working memory, and then a week later, contextual memory was tested. Results revealed that HIV-1Tg rats given a saline injection showed spatial working ($p < 0.01$) and contextual memory deficits ($p < 0.01$) compared to the F344 control group. The chronic nicotine administration significantly improved the deficits of the HIV-1Tg rats in both spatial and contextual memory but has no effects on the contextual learning of the F344 rats. These findings represent a differential effect of nicotine on hippocampal-dependent spatial learning and amygdala-dependent aversive learning in HIV-1Tg rats with impaired memory and in F344 control rats with normal memory. This suggests that nicotinic modulation of neural circuits related to learning and memory could be a target pathway for developing new therapeutic drugs against HIV-associated cognitive impairment. Supported by R01 DA026356 and K02 DA016149

Effects of HIV-1 Tat on enteric neuronal excitability

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About 34 million people are living with the human immunodeficiency virus (HIV) worldwide with about 5 new cases every minute (UNAIDS, 2011). Gastrointestinal (GI) disease has been recognized as a major manifestation of both acute and chronic HIV infection. Since the enteric nervous system regulates GI processes, it may be a potential target of HIV infection. The majority of HIV effects are not achieved by lytic propagation of the virus but by viral proteins. HIV-1 Tat, a regulatory protein between 86 to 101 amino acids is released by infected cells and together with other HIV proteins has been shown to modulate neuronal function in the central nervous system. However little is known about its effects on the GI tract. In this study, we examined the effects of Tat on single isolated enteric neurons and in isolated ileum tissue. At the cellular level, in whole cell patch clamp experiments, Tat increased enteric neuronal excitability both in Tat treated ($n=20$) and in ileum neurons isolated from mice expressing the tat gene ($n=9$) by a significant increase in the number of multiple action potentials evoked, decrease in rheobase (from 20pA to 10pA) and threshold potential (from -13.2mV to -16.1 mV). This increase was mediated by a leftward shift of the sodium channel activation curve (-10mV decrease in $V_{0.5}$ of activation) thereby increasing the steady state availability of sodium channels. Tat selectively increased the mRNA and pro-inflammatory cytokines and increased GI motility thereby contributing to the HIV mediated GI dysmotilities observed in HIV patients. Supported by National Institute of Health grants DK046367 and DA024009 and the VCU-IMSD 541332

Assessment of cannabinoids use on the genital environment: implications for HIV transmission

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Cannabis, more commonly known as marijuana, is the most common illicit drug used and is frequently used in the HIV-infected population, either recreationally or medicinally. Delta-9-tetrahydrocannabinol (THC), the primary psychoactive component of marijuana, has both neurobehavioral and immunomodulatory effects. The specific effects of THC on the genital compartment are unknown as well as the implications for HIV acquisition or transmission. Using the SIV-infected female macaque as a model of HIV disease, we assessed the impact of chronic THC on the vaginal inflammatory milieu and viral shedding. A cohort of 8 female rhesus macaques (4-6 yrs old) was divided into two groups (THC/SIV+, VEH/SIV+) that received chronic THC (0.18-0.32 mg/kg i.m., 2 X daily) or vehicle as a control, starting 28 days prior to i.v. inoculation with SIVmac251. Animals were monitored until they reached end stage disease criteria. The menstrual cycle (average 35 days) was unaffected by THC

administration. Throughout the disease course, the number of inflammatory cells and cytokine levels in vaginal fluids were similar between THC- and VEH-treated animals. Vaginal viral shedding was detected in approximately 60% of samples irrespective of treatment with an average level of 2 log copies/mL observed in both groups. Additionally, plasma viral levels were not significantly different at viral set point (5.8 vs. 5.5 log copies/mL, THC and VEH, respectively). Chronic THC administration did not result in adverse effect the genital milieu in SIV-infected female macaques. Supported by NIDA R01 DA020419; NIAAA 5T32AA007577

HIV-1 Tat mediated induction of IL-6 and IL-8 from astrocytes involves NF- κ B, AP-1 transcription factors and PI3K/Akt, p38 and JNK MAPK signaling pathways

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HIV-1 Tat has been implicated in HIV associated neurotoxicity that is mediated through production of proinflammatory cytokines and oxidative stress. The HIV-1 Tat-mediated production of proinflammatory cytokines includes over expression of IL-6/IL-8 by astrocytes. However, the underlying mechanism(s) are not clearly understood. The present study was designed to determine the mechanism(s) responsible for IL-6 & IL-8 up-regulation. SVGA astrocytes were transiently transfected with plasmid encoding HIV-1 Tat, and IL-6 & IL-8 expressions were measured with peak IL-6 and IL-8 RNA and protein at 6h and 48h post-transfection, respectively. The mechanism(s) were identified by using pharmacological antagonists and siRNA for different intermediate steps involved in PI3K/Akt, p38-MAPK & JNK MAPK pathways. Appropriate controls were used in the experiments and effect of pharmacological antagonists and siRNA were observed on both mRNA expression and protein levels. Our studies indicate that both NF- κ B and AP-1 transcription factors are involved in IL-6 and IL-8 synthesis; however, AP-1 was differentially activated for either cytokine. In case of IL-6 p38 δ activated AP-1 whereas JNK was involved in AP-1 activation for IL-8 production. PI3K/Akt and p38 MAPK (β subunit) were found to be involved in activation of NF- κ B that led to both IL-6 and IL-8 production. Our results present new therapeutic targets that could be used in management of HAND. Supported by DA025528, DA025011, AA020806

Chronic Stress-Induced Blood-Brain Barrier Damage Produced by NMDA Receptor Activation is Associated with Hyper-ramified Microglia

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The blood-brain barrier (BBB) maintains brain homeostasis and protects the brain from potentially harmful, large and non-lipophilic molecules. The putative causes of BBB disruption include increases in glutamate and activation of microglia, which happen to also be affected by stress. However, no studies have investigated the long-term effects of stress on BBB or the mechanisms by which stress causes BBB disruption. Therefore, we examined whether exposure to 21 days of chronic unpredictable stress (CUS) disrupts BBB structure and function and hypothesized that NMDA receptor (NMDAR) activation mediates CUS-induced BBB disruption and microglial activation. Male rats were exposed to 21 days of CUS or handled daily, and pretreated with 0.3 mg/kg MK-801 or saline prior to each stressor. CUS increased NMDAR expression in brain capillaries and microglia branch length, 1 day after the last stressor. CUS also decreased tight junction proteins, claudin-5 and occludin, and increased the extravasation of 10 kDa FITC-dextran into the brain parenchyma, 7 days after CUS. These CUS-induced changes were prevented when rats were pretreated with MK-801. We conclude that stress produces an enduring disruption of BBB structure and function through the activation of NMDAR, and is associated with microglial hyper-ramification. The specific role of hyper-ramified microglia in CUS-induced BBB disruption is being investigated. As stress is comorbid with many neurological diseases, stress-induced BBB damage could significantly affect the progression and extent of the disease process. Supported by NIH Grant: DA035499

Central induction of the HIV-1 regulatory protein, Tat, promotes steroid-sensitive anxiety-like behavior among male and female mice

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Aims: The neurotoxic HIV-1 regulatory protein, transactivating transcriptor (Tat), impairs cognition and promotes gray matter reduction in limbic brain regions of mice. As such, actions of Tat may influence HIV-1-induced behavioral dysfunction. Sex steroids also influence mood, are neuroprotective, and interact with Tat in vitro to ameliorate neurotoxicity. We hypothesized that in vivo expression of Tat would elevate anxiety-like behavior of male and female mice and endogenous or exogenous steroid exposure would ameliorate these effects. **Methods:** GT-tg bigenic mice, which express doxycycline (Dox)-driven Tat protein, were treated with saline or Dox for one week. (1) Proestrous or diestrous female mice were yoked to male counterparts, or (2) ovariectomized mice were

co-administered oil, estradiol and/or progesterone, and were assessed for anxiety-like behavior (open field and marble burying tasks). Results: Among males and females, induction of Tat protein increased anxiety-like behavior across anxiety tasks. Endogenous steroid elevations on proestrous reduced basal anxiety-like responding of females compared to diestrous performances, but were not enough to ameliorate Tat-induced anxiety. Exogenous progesterone, but not estradiol, ameliorated basal and Tat-induced anxiety-like behavior. Conclusions: Exposure to Tat protein may mediate neuropsychiatric sequelae (increased anxiety) associated with HIV-1 infection. Moreover, progestogens may modulate Tat (and by extension, HIV)-related behavioral pathology. Supported by National Institute of Mental Health (MH085607)

Assessment of the gut-mucosal barrier in adult HIV-1 transgenic rats

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Modern day therapy for HIV/AIDS is exceptionally successful at controlling the virus in most organs of the body, but latent reservoirs of HIV remain in some organs, including the gastrointestinal (GI) tract. Thus, the GI tract remains vulnerable to the toxic effects of HIV-1 proteins and HIV-associated inflammation. To enhance understanding of the gut-mucosal consequences of HIV/AIDS, we are studying a rodent model of human HIV infection on combined antiretroviral therapy (cART), HIV-1 transgenic (Tg) rats. The effects of HIV proteins on the function of the gut-mucosal barrier were determined by measuring urine excretion of orally gavaged sugars (sucrose, mannitol, lactulose and sucralose) in adult male Tg and non-Tg Fischer 344 rats. Intestinal permeability was measured by changes in the sucralose/lactulose ratio. This ratio was increased in Tg rats compared to non-Tg rats, indicating a compromised gut-mucosal barrier function. To determine if the leaky gut was associated with disruption of gut-mucosal barrier integrity, we measured expression of the tight junction protein claudin 1 using immunofluorescence. Claudin 1 staining was decreased in the distal colons of Tg rats compared to non-Tg rats, indicating that compromised barrier function may relate to break down of tight junction protein homeostasis. These are ongoing studies, and additional markers will be discussed in the poster. Supported by Supported by USPHSG DA033882; the Chicago D-CFAR P30A1082151; and the Center for Compulsive Behavior and Addiction

Astrocytes exert an anti-viral effect through Wnt secretion

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HIV mediated neuropathogenesis is a multifaceted process involving resident brain cells (neurons, astrocytes, microglia) and infiltrating cells (peripheral blood mononuclear cells (PBMCs)). We evaluated the dynamic interaction between astrocytes and infiltrating PBMCs as it impacts HIV in the CNS. We demonstrate that primary-derived astrocytes (PDAs) secrete Wnt 1, 2b, 3, 5b, and 10b, with Wnt 1 as the predominate Wnt secreted from PDAs. Wnts are small secreted glycoproteins that initiate either Beta-catenin- dependent or independent signal transduction cascade. We show that HIV infection of astrocytes alters this Wnt profile by elevating Wnts 2b and 10b and decreasing Wnts 3 and 5b. PDAs conditioned media (ACM) inhibited HIV replication in PBMCs by 50%. Removal of Wnts from ACM abrogated its ability to suppress HIV replication in PBMCs. Inversely, PBMC supernatant activated PDAs, as demonstrated by a 10 fold increase in HLA-DR and a 5 fold increase in IFN γ expression, and enhanced astrocyte susceptibility to HIV by 2-fold. Collectively, these data demonstrate a bi-directional effect of astrocytes and PBMCs, whereby astrocytes use Wnts as an antiviral factor to suppress HIV replication in PBMCs and PBMCs in turn secrete soluble factors that activate astrocytes, enhance their expression of IFN γ , and enhance their susceptibility to productive HIV infection. This work is supported by R01NS060632-LA and F32NS08065701-MR. Supported by National Institute of Neurological Disorders and Stroke

Cystatin B inhibits the STAT-1 activation in HIV infected or IFN- β induced macrophages

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HIV epidemic remain prevalent. Current efforts are directed to deplete HIV from lymphocyte and macrophage reservoirs. Previous studies from our group discovered that cystatin B, a widely cysteine protease inhibitor, induces HIV replication in monocyte-derived macrophages (MDM) and interacts with STAT-1. Studies with luciferase reporter gene assays in Vero cells determined that cystatin B inhibited the IFN- β response by preventing STAT-1 translocation to the nucleus and decreasing levels of tyrosine phosphorylated STAT-1 (STAT-1PY). In order to understand the role of cystatin B in HIV replication in macrophage reservoirs, this study aims to elucidate if this protein regulates STAT-1 activation in IFN- β induced macrophages and its effect in HIV-replication. Cystatin B siRNA was transfected into MDM prior to infection with HIV-ADA and cultures were followed for 12 days.

Immunofluorescence and Proximity Ligation Assay were used to determine the effect of cystatin B modulation in STAT-1 localization and activation. We found that both, HIV infection and IFN- β addition to MDM, induced intracellular expression of cystatin B and cystatin B/STAT-1 interaction. However, STAT-1 was retained in the cytoplasm. Cystatin B siRNA treatment decreased viral replication and increased STAT-1PY in HIV infected MDM. Upon IFN- β induction, MDM silenced for cystatin B allowed STAT-1 translocation to the nucleus. These data suggest that cystatin B inhibits IFN- β anti-viral responses and induces HIV replication in macrophage reservoirs through the inhibition of STAT-1 activation. Supported by NIH NIMH 5F32MH094210-02, NIH NINDS-SNRP-U54NS4301, NIH R01MH083516

Increased cortisol levels correlate with depressive symptoms in HIV positive individuals

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Cortisol is a glucocorticoid secreted by the adrenal cortex and is used as a marker of stress. Cortisol levels can increase with age, HIV/AIDS and major depressive disorder. We want to study the effects of depression on the cortisol levels, which remain unknown in HIV-infected Puerto Ricans. We hypothesize that depression will lead to increased cortisol levels in this population. In our analyses we included males and females > 21 years, HIV sero-status and plasma viral load having antiretroviral treatment and regimen. Patients completed the participation consent, socio-demographic information, and Patient Health Questionnaire-9 (PHQ-9) for depression assessment. We collected blood samples from each participant for further centrifugation and isolation of plasma layer for the quantification of cortisol levels according to the manufacturer's protocol (Cayman chemicals). According to our results HIV+ patients with depression have higher cortisol levels when compared with those patients without depressive symptoms. Supported by Ponce School of Medicine and Health Sciences Institutional Funds, NCRR (P2ORR016470), NCRR (RR003050), and NIMHD (G12-MD007579)

CD8 T cells modulate NSC proliferation by modulating macrophage activation during Herpes Simplex encephalitis

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Herpes Simplex encephalitis is characterized by chronic inflammation that is dominated by CD8 T-cells. Previous studies have demonstrated that neural stem/progenitor cell (NSC) proliferation peaks at 6 dp.i. followed by a significant reduction in proliferation at 15 dp.i. Peak NSC proliferation occurred concurrent with M2 macrophage infiltration. At 15 dp.i., M1 activation markers were predominantly observed and approximately 50% of infiltrating CD8 T-cells produced virus-specific interferon- γ . CD8 T-cell depletion in the brain at 15 d p.i resulted in a 5-fold increase in nestin(+) NSCs compared to isotype-antibody-treated controls. While CD4(+) T cell numbers or activation did not change significantly, an increase in the total number of infiltrating macrophages (CD45(hi)CD11b(+)) as well as a down-regulation of MHCII on microglia was observed. Interestingly, infiltrating macrophages in CD8 depleted mice had a 3-fold greater expression of CD206, an M2 macrophage marker. Further analysis of macrophage phenotypes showed that, in the absence of CD8 T-cells, the ratio of CD206 to CD86 macrophages shifted from 2:1 to approximately 4:1, suggesting that CD8 depletion promotes M2 macrophage activation in the brain. Gene transcription analysis demonstrated a significant reduction in IFN- γ production in depleted mice. Taken together, these data suggest that T cell produced IFN- γ mediates impairment of NSC proliferation by altering brain macrophage activation. These studies may help identify therapeutic interventions to enhance neurogenesis during viral encephalitis. Supported by NIH NIDA T32 DA007097 and NINDS RO1 NS065817

Novel magneto-electric nanodelivery of "microRNA mimic" across blood-brain barrier: Implications to cocaine modulation on HIV-associated neurocognitive disorders

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The "miRNA mimics/inhibitors" strategy is one of the emerging fields in therapeutics of many diseases. Our laboratory has shown significant downregulation of miRNA-155 during HIV infection and/or cocaine exposure and subsequently, transfection with "miRNA-155 mimic" remarkably inhibits the viral replication. Thus, delivery of "miRNA-155 mimic" in CNS could be beneficial in treatment of HIV-associated neurocognitive disorders. Nevertheless, impermeability of most exogenous molecules across blood-brain barrier (BBB), together with the lack of efficient technology, is major hurdle in CNS delivery of therapeutics. We herein report application of recently

discovered multifunctional magneto-electric nanocarrier (MEN) for delivery of “miRNA-155 mimic” across BBB. Transmission electron microscopy was used to characterize the MEN and “MEN-miRNA mimic” nanoformulation was confirmed by fluorescent-based detection assay. Nearly 50% miRNA binding on MEN was achieved in 1 hour. Application of a very low AC field (65 Oersted at 0 Hz) resulted in nearly 100% release of bound miRNA from the MEN. More importantly, influence of remote low-energy DC magnetic field resulted in ~ 25% higher miRNA translocation across in vitro BBB compared to free miRNA ($p < 0.0016$) within 2 hr. Applying ME field did not alter the BBB integrity and cell viability. Further, MEN is being evaluated for their neurotoxicity and neuro-behavioral modulating ability in mouse model. Thus, ME nanocarrier could be used for speedy, targeted, and controlled delivery of drugs in CNS. Supported by NIH

HIV-1 gp120 induces unfolded protein response (UPR) via endoplasmic reticulum (ER)-stress genes: Role in astrocyte toxicity.

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HIV-1 gp120 is known to play an important role in HIV-mediated neurotoxicity, which is largely associated with proinflammatory cytokines/chemokines and oxidative stress. The UPR-mediated apoptosis has been implicated in several neurodegenerative diseases; however, its role in HIV/neuroAIDS remains unexplored. The present study was undertaken to assess the possible involvement of UPR via ER-stress genes for HIV gp120-mediated apoptosis. The SVGA astrocytes were transfected with gp120 and the expressions of ER-stress genes were measured. HIV-1 gp120 induced GRP78/BiP and CHOP by 3.4 ± 0.2 fold and 6.1 ± 1.0 fold, respectively at mRNA and 1.3 ± 0.2 fold and 2.2 ± 0.4 fold, respectively at protein levels. The UPR pathways such as ATF6, IRE1 α and PERK pathways were investigated to explain a possible mechanism and were found to operate simultaneously as evident by golgi-specific localization of ATF6, alternative splicing of XBP-1 and induction of p-eIF2 α and ATF4. This was further confirmed using siRNA for ATF6, IRE1 α and PERK, which showed > 50% reduction in CHOP expression. Furthermore, siRNA against IRE1 α and PERK but not ATF6 rescued the cells from cell death when measured using MTT assay suggesting their roles in gp120-mediated apoptosis. Altogether, the present study is the first report demonstrating the involvement of ER-stress in gp120-mediated apoptosis and its mechanistic details. In future, the role of UPR via ER-stress in gp120-mediated cell death could present novel therapeutic targets for treatment of neuroAIDS. Supported by DA025528, DA025011, AA020806

Morphine modulation of TLR2 induced IL17A release in bronchial epithelial cells in the context of HIV infection

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Pulmonary inflammation in HIV patients due to opportunistic infections is poorly studied phenomenon. Chronic morphine cause persistent inflammation, which is attributed to mainly TLR2 and its stimulation triggers NF- κ B mediated IL17 production. HIV-1 infection, chronic morphine, TLR2 and IL17R signaling represent a single network, where one controls the other to disrupt the balance, leading to uncontrolled inflammation. We focus on the role of bronchial epithelium and the synergism between TLR2 and IL17R signaling pathways and how morphine and EcoHIV disrupt homeostasis. Our studies showed, 16HBE14o cells constitutively express IL17A within the cells, which was detected by western blot and flow cytometry. In In-vivo, TLR2 mediated IL-17A release is an early innate response, which is diminishes by 24h and morphine dampens this early response but sustains an elevated IL17A at later time points. The early release of IL-17A is a TLR2 stimulated secretion rather than synthesis. We showed, MyD88 and Act1 associate with their respective receptors and with each other within 30min of TLR2 stimulation, which is disrupted by morphine, leading to uncontrolled pro-inflammatory signaling. Studies are in progress to identify TLR2 and IL17R signaling intermediates, which interact with each other to beget TLR2 tolerance, thereby identifying novel therapeutic target. Finally, we will apply intervention studies, where blockade of TLR2 and/or IL17R will be monitored as a potential therapy to reduce morphine induced prolonged inflammation in the context of opportunistic infections and HIV. Supported by RO1 DA 12104, RO1 DA 022935, RO1 DA031202, K05DA033881, P50 DA 011806, 1R01DA034582

Implications of astrocytic Nrf2-ARE signaling pathway in Methamphetamine and HIV-1 gp120 induced oxidative stress

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Despite the advent of the antiretroviral therapy, HIV-1 associated neurocognitive disorders (HAND) continue to be a significant issue for HIV-1 infected patients. In HAND, astrocytes fail to protect themselves from oxidative stress mediate damage resulting in apoptosis. NF-E2-related factor 2 (Nrf2) transcription factor plays vital role in cellular protective response against oxidative stress due to environmental agents such as electrophiles, drug abuse, smoking, radiation. Although HIV-1 accessory proteins and Methamphetamine (METH) has been implicated in the pathogenesis of HAND, little is known about their combined effect on the regulation of Nrf2 in human astrocyte. In this study, we investigated the combinatorial effect of HIV-1 accessory proteins and METH on Nrf2-ARE signaling pathway in human primary astrocytes. Escalating dose of METH and gp120 induced astrocyte activation as observed by GFAP immunocytochemical staining. It also caused significant increases in protein carbonylation and DNA fragmentation as compared to controls. More importantly, METH and gp10 significantly increased Nrf2 phosphorylation and nuclear translocation in a time dependent manner, which is associated with upregulation of key antioxidant enzymes. Taken together, these results suggest the involvement of PKC-dependent Nrf2-ARE signaling pathway as a protective response to METH- and gp120- induced oxidative stress in human astrocytes.

The role of Ankyrin- rich membrane spanning protein (ARMS/Kidins220) in Human Immunodeficiency Virus type 1 (HIV-1) induced microglial activation.

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Microglia are CNS resident immune cells that are directly infected by HIV-1, and consequently secrete multiple proinflammatory molecules contributing to HIV-1-induced neuroinflammation. Activation of microglial cells is believed to occur due to actions of viral factors (mainly Tat) that are secreted by infected cells in the CNS. Ankyrin-rich membrane spanning protein (ARMS/Kidins220) is a multidomain transmembrane protein that is involved with neurotrophin signaling in neurons, and also plays an important role in nervous system development. We have previously established the role of ARMS in linking NF- κ B activation and neurotrophin signaling in these cells. Recent reports also have suggested a non-neuronal role for ARMS in multiple immune cell types. In this study we demonstrate that ShRNA mediated ARMS knockdown was associated with marked reduction in the HIV-1 Tat-induced proinflammatory response, including TNF- α production and NF- κ B activation. In the absence of ARMS we also observed stabilization of inhibitory kappa B (I κ B α) in response to Tat exposure, thus indicating that Tat-induced NF- κ B activation is dependent on normal expression of ARMS. Furthermore, co-immunoprecipitation studies suggest that ARMS physically interacts with inhibitory kappa B kinase (IKK) subunits in order to activate NF- κ B signaling. Our results strongly establish the role of ARMS in microglial activation by HIV-1 Tat. These findings with further investigations may uncover novel therapeutic targets for the treatment of HIV associated Neurocognitive Disorders. Supported by (NIH grants RO1 NS054578 and RO1 NS066801) to Dr. Sanjay Maggirwar.

Tumor necrosis factor- α confers cytotoxicity in astrocytes under oxidative stress via inhibition of NF- κ B signaling

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Oxidative stress and inflammation together recognized as central feature of both acute and chronic neurological disorders. In acute ischemic stroke, formation of H₂O₂ causes brain injury, which appear to be exacerbated by IL-1 β or TNF- α produced after reperfusion. However, evidences also show that TNF- α helps in recovery and repair. Therefore, role of TNF- α is unclear. Further, it is unknown how astrocytes are affected when oxidative stress and inflammation coexist. Here we examined the effects of H₂O₂ on cell survival and NF- κ B dynamics in cultured human astrocytes co-stimulated with TNF- α or IL-1 β . Data showed H₂O₂-treatment significantly increased apoptosis in astrocytes in dose-dependent manner; however, IL-1 β or TNF- α -alone did not. Interestingly, co-treatment of TNF- α , but not IL-1 β with non-toxic dose of H₂O₂ significantly increased apoptosis in astrocytes. The toxicity of co-treatment of TNF- α and H₂O₂ was significantly higher than respective dose of H₂O₂-alone. Investigations of mechanisms revealed that H₂O₂ inhibited TNF- α -induced translocation of NF- κ B to the nucleus in astrocytes thereby inhibiting cellular defense and/or survival pathways. H₂O₂ decreased level of TNFR1 associated protein, RIP1, necessary for I κ B kinases activation, thereby inhibited I κ B- α degradation and NF- κ B nuclear translocation. This supports the evidence of H₂O₂ as a modulator of pro-inflammatory signaling and explains the increased sensitivity of astrocytes during brain injury. These data also signify need to design strategy to combat oxidative stress during neuroinflammation and repair. Supported by NINDS/RO1 NS48837 and RO1 NS070896-01

Aging synaptic mitochondria exhibit dynamic proteomic changes: applications for the study of mitochondrial alterations associated with an aging HIV+ population.

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As HIV+ individuals are living longer, the impact of aging on their progressive neurocognitive decline is an emerging challenge. The aging brain is characterized by a shift to a proinflammatory state, which likely exacerbates the neuroinflammation and synaptodendritic degenerative changes in HIV, which correlate with the presence and severity of cognitive impairment. In an aging HIV+ population, mitochondria may be a critical link as their dysfunction is a constant finding in both inflammatory and neurodegenerative conditions. Increasing evidence implicates mitochondrial dysfunction as a major player leading to synaptic failure. Therefore, we examined the relationship between aging, synaptic mitochondrial dysfunction and alterations in synaptic mitochondrial protein expression. We developed a super-SILAC method for quantitative proteomics of mouse brain mitochondria and accurately quantified the proteome differences of synaptic mitochondria isolated from 5, 12, and 24-month old mice. Altered protein expression levels were verified orthogonally with Western blot focusing on those enriched in pathways relevant to HIV-associated mitochondrial dysfunction including mitochondrial bioenergetics and dynamics. The proteomic results combined with bioenergetic functional analysis revealed distinct alterations in synaptic mitochondria during aging and set the stage to study such alterations in the presence of HIV. We believe this study provides a framework for future work that will lead to improvements in the treatment of neurocognitive deficits in both age-related diseases and HIV. Supported by NIH CHAIN Grant

Role of Proline Metabolism in Exacerbating Psychostimulant Induced Neurotoxicity in HIV-associated Neurological Disorder

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Cocaine is a commonly used drug among HIV patients and has been suggested to worsen HIV-associated neurocognitive disorders (HAND). We propose a novel mechanism mediated by the cellular enzyme "Prolidase" in cocaine and HIV-1 induced neuronal damage. Prolidase plays a critical role in the turnover of collagen present in the basal lamina of the blood-brain barrier (BBB). Notably, collagen is a reservoir of the secondary amino acid "proline" and higher level of proline in the brain causes neuronal dysfunction. Since data suggest that both HIV and cocaine disrupt the BBB, this disruption is likely to increase proline levels in the brain. However, a role of proline metabolism in HAND is yet to be elucidated. Therefore, we hypothesize that cocaine together with HIV-1 will increase proline levels in the brain that will induce neurological damage/dysfunction. To test this, we treated human brain microvascular endothelial cells (hBMVECs) with HIV-1 gp120 and cocaine and examined the expression and activity of Prolidase and the Matrix metalloproteinases (MMPs)-the enzymes that are important for the degradation of collagen. We found that cells treated with both cocaine and HIV-1 gp120 have substantially higher expression and activity of prolidase and MMPs compared to individual treatment of cocaine or HIV-1 gp120. Thus, we envision the altered balance in proline homeostasis at the BBB can leave the neurons vulnerable for damage and dysfunction. This phenomenon may partly account for the reason why HIV positive individuals who also abuse cocaine have increased pathogenesis of HAND. Supported by NIH

Anti-HIV-1 miRNAs Regulate Cocaine Induced Enhancement of HIV-1 Replication in Human Monocyte Derived Macrophages

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The prevalence of HIV- associated neurocognitive disorders (HANDs) is increasing among HIV-1 infected individuals. Caused by HIV infection in the brain, HANDs are a result of subsequent inflammation and damage within the central nervous system (CNS). Macrophages are the primary target for HIV replication in the CNS and have been shown to play a major role in HAND pathogenesis. Furthermore, cocaine, a commonly used drug among HIV patients has been suggested to worsen HAND. While studies demonstrate increased HIV replication in monocyte derived macrophages (MDMs) by cocaine, the mechanisms for this enhancement are not completely understood. We propose a novel mechanism mediated by the cellular anti-HIV-1 miRNAs for the potentiating effects of cocaine on HIV-1 replication in MDMs. This is based on our data illustrating that cocaine down-regulates the anti-HIV-1 miRNAs miR-125b, miR-150, and miR-28-5p in primary MDMs. Since these miRNAs are known to target the HIV-1 mRNA and inhibit viral translation, we hypothesize that cocaine-induced down-regulation of these miRNAs increases HIV replication in MDMs. Therefore, the goal of this study is to examine whether down-regulation of these miRNAs directly increase HIV-1 replication in MDMs. Our strategy is to abrogate cocaine's effect with complementing miRNA-mimics and simulate cocaine's effect with complementing anti-miRNAs. We envision that this mechanism of enhanced HIV replication may identify cellular factors/pathways that can be

targeted for therapeutic intervention of cocaine abusing HIV-1 infected patients. Supported by T32 grant #2T32H007735-17 from NIH/NHLBI and DA024558, DA30896, DA033892 from NIDA/NIH to CD.

Quantitative and qualitative assessment of monocyte trafficking into inflamed brain tissue

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Mononuclear phagocytes (MP; monocytes, macrophages and dendritic cells) serve as a first line of immune defense, as disease perpetrators, and as carriers of drugs and genes for therapeutic delivery. MP may be particularly useful for delivering large molecules into the injured central nervous system (CNS), across the blood brain barrier (BBB). To quantitatively assess MP brain entry we adoptively transferred bone marrow-derived monocytes (BMDM) via the intravenous route into C57BL/6 mice with acute CNS inflammation triggered by intracranial injection of *E. coli* lipopolysaccharide (LPS). We then examined parameters that may influence the efficiency of monocyte trafficking into the CNS, and found that prolonged in vitro culture of monocytes negatively affects their ability to enter the brain. In contrast, cell uptake was enhanced under conditions of greater inflammation. We also examined the persistence and phenotype of the exogenous monocytes that entered the brain, and demonstrated that transient disruption of the blood-brain barrier by mannitol, bradykinin and serotonin could increase the efficiency of cell delivery to the CNS. These findings have relevance to the use of monocytes as a vehicle for delivering therapeutic "cargo" to the CNS, for treatment of neurodegenerative disorders. Supported by the National Institute of Mental Health and National Institutes of Health (R01 MH079717)

Astrocyte expression of HIV-1 Vpr in the hippocampus induces structural changes in neurons and learning impairment

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HIV infected individuals are at an increased risk of developing neurological abnormalities. HIV induces neurotoxicity by host cellular factors and individual viral proteins. Some of these proteins including Viral Protein R (Vpr) promote immune activation and neuron damage. Vpr is known to contribute to cell death of cultured rat hippocampal neurons and suppresses axonal growth. Thus Vpr may play a role in hippocampal loss of function. In this study we demonstrate that HIV-1 Vpr produced by astrocytes in the hippocampus impairs hippocampal-dependent learning. Live rats were studied by the novel object and novel location tasks after hippocampal infusion with astrocytes expressing HIV-1 Vpr. Our results showed impaired novel location and novel object recognition compared with control rats expressing green fluorescent protein (GFP). This impairment was correlated with a significant decrease of synaptophysin immunoreactivity in hippocampal CA3 region, suggesting synaptic injury in HIV-1 Vpr-treated animals. Patterns of nissl staining showed morphological changes indicative of neuronal chromatolysis in the Vpr group. These findings of Vpr-induced changes in neuronal morphology and synaptic injury may explain the findings of neuronal dysfunction of spatial and recognition memory deficits, not present in control rats. The data suggest Vpr as a neurotoxin with potential to cause learning impairment in HIV-1 infected individuals even under conditions of limited viral replication. Supported by R03DA026722 and G12RR003050

HIV-1 gp120 affects the expression of the glutamate-glutamine cycle proteins in human astrocytes

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HIV-1 infection can cause immunodeficiency and HIV-Associated Neurocognitive Disorders (HAND) in subjects on antiretrovirals. Viral proteins within the central nervous system (CNS) may worsen HAND by altering glial glutamate metabolic control. Astrocytes regulate the glutamate-glutamine cycle (GGC) by controlling the protein expression and metabolic activities of its enzymes. Glutaminase (GLS) and Glutamate Dehydrogenase (GDH) produce glutamate whereas Glutamine Synthetase (GS) lowers glutamate levels. Glutamate transporters EAAT-1/2 clear extracellular glutamate and are decreased by HIV-1 gp120 allowing its build up. However, whether HIV-1 gp120 can impair glutamate metabolism by increasing GLS or GDH levels is unknown. We hypothesize that astrocytic expression of HIV-1 gp120 will impair the expression pattern of glutamate regulatory proteins. We transfected U87MG astrocytes with either mock or HIV-1-gp120 expression vectors and evaluated their GGC protein expression at time point analyses. Proteins were analyzed by Western Blot and enzyme activity determined with a GDH detection assay. Densitometry values were adjusted to each loading control and statistically analyzed by one-way ANOVA with post-hoc Tukey-Kramer test. We expect that astrocytic expression of HIV-1 gp120 decreases GS, EAAT-1/2 protein levels and increase GLS and GDH levels when compared to control astrocytes. Our results will determine whether astrocytic HIV-1gp120 promotes neurotoxicity by affecting GGC proteins,

providing new understanding about glutamate toxic mechanisms that worsen HIV neuropathology. Supported by NCRR-RR003050/NIMHD-G12-MD007579, NCRR-P20RR016470, R25GM08420

Mitochondrial abnormalities present in the HIV-1 transgenic rat

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Human immunodeficiency virus-1 (HIV-1) infection can produce neurocognitive impairment in infected individuals despite the lack of a productive infection in the brain. Unfortunately, the cause of this impairment remains unclear. Several lines of evidence implicate mitochondria in the pathology. Such evidence includes an increase in oxidative stress, depleted energy stores, increased apoptosis and increased prevalence of mitochondrial DNA deletions in HIV-1 infected individuals. Furthermore, the HIV-1 viral proteins Gp120, Nef, Tat and Vpr have been shown to detrimentally interact with mitochondria. To investigate the neurological component of HIV-1 infection, we chose to study the neuronal mitochondria in the HIV-1 transgenic rat. This rat model expresses every gene from the HIV-1 provirus except Gag and Pol throughout the body. Previous work has demonstrated that this model displays several functional neurological abnormalities similar to HAND in HIV-infected humans, including aging effects and interactions with drugs of abuse. Using stable isotope labeling by amino acids in cell culture (SILAC)-based proteomics, we were able to identify mitochondrial proteomic alterations in the transgenic animals. Biological processes involved in synaptic signaling, exo- and endocytosis, and neuron development were altered. This research suggests mitochondrial may have a larger role in the HIV-1-associated neuropathogenesis than previously expected.

Morphine disrupts gut microbiota and promotes systemic dissemination of *Citrobacter rodentium*

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Gut microbiome plays an important role in maintaining gut homeostasis. However, the effect of morphine on gut microbiome has not been investigated. *Citrobacter rodentium* is a natural mouse pathogen that models intestinal infection by enteropathogenic *Escherichia coli* (EPEC) and enterohemorrhagic *E. coli* (EHEC) in humans and causes attaching and effacing (A/E) lesions and colonic hyperplasia. This study examined the effects of morphine treatment on the mouse colonic microbiome and dissemination of *C. rodentium* following infection. Colonic contents of mice were extracted and examined using Illumina 16S rRNA metagenomic sequencing and PhyloChip microarray analysis. Operational taxonomic unit (OTU) analysis and Principal Coordinate Analysis (PCoA) indicated a significant shift in the colonic microbiome following morphine treatment when compared to placebo. The differences in microbiome clustering were greater in the context of infection with *C. rodentium*. A number of genus-level enrichments and depletions were observed in response to morphine treatment alone, or morphine in combination with *C. rodentium* infection. Of note, OTU 971 was more abundant in morphine samples when compared to placebo samples and OTU 499 was more abundant in the placebo samples. The most notable effect observed in this study was that morphine treatment resulted in increased systemic dissemination of *C. rodentium*. This is the first study to demonstrate that morphine modulates gut microbiome and promotes pathogen dissemination in the context of *C. rodentium* intestinal infection. Supported by RO1 DA 12104, RO1 DA 022935, RO1 DA031202, K05DA033881, P50 DA 011806, 1, R01 DA034582 (P.I. S.R.)

Mitogenic growth factors induce proliferation of neural progenitor cells through YAP activation

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Neural progenitor cells (NPCs) exist in the developing and adult brain and are able to generate new neurons. NPCs are routinely studied in vitro using neurosphere culture and mitogenic growth factors, including epidermal growth factor, basic fibroblast growth factor, and Insulin-like growth factor 1. Yes-associated protein (YAP), a main effector of the Hippo signaling pathway, has recently been identified as a key factor regulating NPC proliferation. However, the upstream signaling that activates YAP in NPCs remains unclear. We hypothesize that the mitogenic growth factors regulate NPC proliferation through YAP activation. To test the hypothesis, we treated NPCs with individual growth factor and identified YAP activation through Western blotting and immunocytochemistry. We found that mitogenic growth factors significantly inhibited YAP phosphorylation and induced nucleus translocation of YAP, both indicative of YAP activation. The YAP activation was consistent with inhibition of phosphorylation of its upstream factors including Mst1 and Lats1, suggesting a systematic signaling change of the Hippo pathway. To

determine the functional importance of YAP activation in NPCs, we used a specific siRNA to silence YAP expression in NPCs and found that lack of YAP dramatically reduced growth factor-induced NPC proliferation. Together, these results suggest that mitogenic growth factors are important upstream signaling molecules for YAP-mediated NPC proliferation. Identification of these signaling pathways upstream of YAP provides new molecular mechanisms to control NPC functions. Supported by R01 NS 41858-01 and R01 NS 061642-01

Activation of VDR attenuates HIV-induced podocyte apoptosis

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HIV-associated nephropathy (HIVAN) is a severe kidney disease characterized by heavy proteinuria, rapidly progressive renal insufficiency, and distinct kidney morphological changes. HIV-induced apoptosis of glomerular visceral epithelial cells (podocytes) is a major reason leading to proteinuria in HIVAN. To test whether activation of vitamin D receptor (VDR) can attenuate HIV-induced podocyte apoptosis, we performed both in vitro and in vivo studies. In vitro study, we added VDR agonist EB1089 to HIV-infected human podocytes, and examined its effect on apoptosis, ROS generation, and associated molecules. We found that addition of EB1089 significantly decreased HIV-induced ROS generation and apoptosis in podocytes. The expression of podocyte markers, including podocin, CD2AP, and WT1, were decreased by HIV, while their expressions were partially rescued by EB1089. EB1089 also increased the expression of longevity gene FoxO3A; while silencing FoxO3A with siRNA abolished EB1089-attenuated podocyte ROS generation and apoptosis. In vivo study, we treated HIVAN mouse model Tg26 mice with vitamin D for 3 weeks, and ELISA results showed that the proteinuria was dramatically decreased when compared with the saline treatment alone. Immunohistochemistry staining displayed decreased apoptosis and increased FoxO3A expression in the podocytes of Tg26 mice administrated with vitamin D. These results indicate that activating VDR can partially protect HIV-infected podocytes through upregulation of FoxO3A, and our study provides insight into new target for HIVAN therapeutic strategy.

Self Protein Specific CD4+ Effector T cell Line Exacerbates MPTP Induced Neurodegeneration in a Mouse Model of Parkinson's Disease

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Inflammation plays a noteworthy role in Parkinson's disease. We have previously shown in the 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine (MPTP) mouse model that T cell-mediated immunity regulates innate immunity to control or exacerbate neuroinflammation. Effector T cells that recognize nitrated α -synuclein (N- α -syn) as non-self increase reactive myeloid microglia and inflammatory mediators with prolonged progression and increased dopaminergic neurodegeneration within the substantia nigra (SN) of MPTP treated mice. This study focuses on the generation of an N- α -syn specific CD4+ T cell line with the ability to increase release of reactive nitrogen species (RNS) from myeloid lineage cells and also intensify the loss of tyrosine hydroxylase (TH) positive neurons within the SN of mice that have been intoxicated with MPTP. Flow cytometric analysis showed the T cell line exhibited very distinct and heavy labeling for CD3, CD4 and IL-17a, moderate staining for CD146, yet very little expression of CD8 and FoxP3. Co-culture of the T cells with N- α -syn and BV-2 cells increased RNS production of the BV-2 cells by 150% compared to controls. Lastly, adoptive transfer of the T cell line to MPTP intoxicated mice exacerbated the loss of TH+ dopaminergic neurons by 63% compared to controls. Together, these results support the notion that effector T cells specific for N- α -syn increase oxidative stress as measured by RNS release from reactive myeloid microglia, increase the loss of TH+ neurons, and thus may play a pivotal role in the tempo and progression of Parkinson's disease. Supported by National Institute of Health 3R01NS070190-03S1 (RAW) and 5R01NS070190-03 (RLM)

Exercise impacts tumor cell trafficking into the brain via modulation of redox status.

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The mechanisms impacting tumor cell extravasation into the central nervous system, and the influence of behavioral modifications remain elusive. Cell extravasation into the brain requires passage through the blood-brain barrier (BBB), and disruption of tight junctions, which provide a protected interface between the brain and the rest of the body. Changes in redox status within the microvascular environment can activate signaling events including redox-sensitive small GTPases, leading to the disruption of the BBB and promoting cell extravasation. In the current study, mice voluntarily exercised for five weeks using a running wheel, and the average voluntary running distance was 9.0 ± 0.3 km/day. The mice were infused with 1×10^6 D122 cells (murine Lewis lung carcinoma) into

brain microvasculature in order to study cell extravasation of circulating cells. Microvessels were isolated 48-hours (short-term) or 2.5 weeks (long-term) post tumor cell administration. Oxidative stress levels were increased in exercised tumor cell infused mice, however there was a significant negative correlation between oxidative stress and running distance. Additionally, Rho activation was negatively correlated with running distance in short-term studies. Exercised mice in long-term studies showed decreased metastatic growth. These data suggest that exercise can influence the initial steps of metastasis formation by modulating redox signaling in the brain microvasculature. Supported by NIH/NCI R0CA133257

Selenium-containing compounds regulate adhesive properties of the brain vascular endothelium and influence early events in brain metastatic progression via a mechanism involving NF- κ B.

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Anticarcinogenic qualities of selenium (Se) have been attributed to strong antioxidative and anti-inflammatory properties. In order to address possible mechanisms involved in these effects, selenoglycoproteins were extracted from Se-enriched yeast at pH 4.0 and 6.5 (SGP40 and SGP65), followed by evaluation of their impact on the interactions of lung and breast tumor cells with human brain microvascular endothelial cells (HBMEC). Extracted selenoglycoproteins, especially SGP40, significantly attenuated adhesion of tumor cells to HBMEC and their transendothelial migration. Because the active component(s) of selenoglycoproteins are unknown, Se-containing fractions of low molecular weight (<1,000 Da), which are normally present in selenized yeast, were introduced as additional treatment groups. Treatment of HBMEC with SGP40 and the isolated fractions induced changes in gene signatures, which suggested a central involvement of NF- κ B. These observations were confirmed in the subsequent analysis of NF- κ B DNA binding activity, quantitative measurements of the expression of selected genes and proteins, and tumor cell adhesion assay following treatment with a specific NF- κ B inhibitor. The findings indicate that specific organic Se-containing compounds have the ability to inhibit early phases of brain metastatic tumor formation via a process, which involves downregulation of NF- κ B. Selenoglycoproteins appear to be more effective than small Se-containing compounds, suggesting a role for not only Se but also the glycoprotein component in the observed effects. Supported by National Institutes of Health CA133257 and Alltech Nutrigenomics

Roles of IP3R2 in Tat-mediated endoplasmic reticulum stress in astrocyte: implication for HAND

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ER stress has recently been implicated to play an important role in the HAND. While Tat-mediated neurotoxicity involving dysregulation of intracellular Ca²⁺ has been well-documented, relatively little is known about how disruption of Ca²⁺ homeostasis is linked to ER stress in astrocytes. Recent data from our lab demonstrates that Tat triggered ER stress in astrocytes as evidenced by activation of PERK/eIF2, ATF6 & IRE1/XBP1. This process involved increases in Ca²⁺ transients, with blockade of the effect using IP3R receptor antagonist. Direct binding of Tat to the IP3R2 receptor was assessed using the GST pull-down assays followed by functional assays using the FRET based Ca²⁺ biosensor. Involvement of Tat-mediated ER stress/ Ca²⁺ dysregulation in cellular activation was validated in IP3R2 knockdown astrocytes, which failed to upregulate inflammatory cytokines following Tat exposure. Further confirmation of these findings in IP3R2 knock out mice is currently ongoing. In vivo implication of these findings was confirmed by microinjecting HIV Tat protein in the striatum of C57BL/6N mice and staining the brain sections for ER stress markers. Studies with IP3R knockout mice are in progress. Taken together our findings provide evidence that Tat disrupts ER Ca²⁺ homeostasis through binding to IP3R2, which, in turn, signals the downstream ER stress pathway leading ultimately to astrocyte activation. Intervening ER stress signaling and or IP3R could be envisioned as potential therapeutic targets for neurodegenerative diseases involving astrocyte activation. Supported by MH-068212, DA020392, DA023397, DA024442

Cocaine Potentiates Cathepsin B Secretion in HIV-infected Macrophages and Contributes to HIV-1 Associated Neuronal Apoptosis

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Drugs of abuse are risk factors for HIV infection and progression to AIDS. Recent evidence establishes that cocaine use promotes brain perivascular macrophage infiltration and microglia activation. The lysosomal protease

cathepsin B is increased in monocytes from patients with HIV dementia and its secretion from HIV-infected monocyte-derived macrophages (MDM) induces 10-15% of neurotoxicity. Here we asked if cocaine induce secretion of cathepsin B from HIV-infected MDM and its effect in neuronal apoptosis. To test this hypothesis MDM were inoculated with HIV-1ADA, exposed to cocaine, and followed for up to 12 days. Serum-free supernatants from HIV-infected cocaine treated MDM and cocaine negative controls were added to neuronal cells at 6 and 12 days post-infection (dpi) and apoptosis measured by TUNEL and analyzed with Image J software (NIH). Cathepsin B concentration and activity as well as cathepsin B inhibitors were measured from the MDM supernatants. Cathepsin B concentration and activity was increased HIV-infected cocaine treated MDM compared to cocaine negative controls. A significant increase of 30% of apoptotic neurons at 6dpi and 12dpi was obtained that was reverted with the specific cathepsin B inhibitor (CA-074) and with cathepsin B antibody. To validate our findings, plasma from HIV/cocaine users and HIV non-drug users were tested for cathepsin B and its inhibitors. Cathepsin B was significantly increased in HIV/cocaine users. Our results demonstrated that cocaine potentiates cathepsin B secretion in HIV-infected MDM and induces neuronal apoptosis. Supported by Sponsored in part by NIH SNRP-NINDS U54NS431, INBRE P20RR016470-12, NIMHHD 8G12-MD007600, R01MH083516, NIGMS RISE R25GM061838

Abstracts Submitted for General Poster Session 2

Cannabinoid Agonists Regulate Alcohol-induced Inflammation in Human Dendritic Cells

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Alcohol dependence (AD) is a complex disorder regulated by multiple mechanisms including the cannabinoid system. For instance, cannabinoid receptor 2 (CB2) stimulation has become a target for neuroprotective therapies; and in the immune system, CB2 is known to have immune-modulatory effects. Recently, we reported that CB2 and GPR55 play a protective role under EtOH-induced inflammation in vitro. Although we demonstrated that EtOH induces cannabinoid receptors (CBR) and they regulate inflammatory cytokine production by monocyte-derived dendritic cells (MDDCs), the regulatory role CB2 and GPR55 agonists (JWH-015 and O-1602, respectively) play on MDDCs treated with EtOH remains unclear. Therefore, it is hypothesized that CBR agonists can regulate cytokine production and EtOH-induced oxidative stress in MDDCs. Therefore, MDDCs were cultured with EtOH (0.1 and 0.2%) and/or CBR agonists for 24-48 hrs. Arrays results show an increased in pro-inflammatory cytokines after EtOH treatment, and treatment with CBR agonists show lower induction of pro-inflammatory cytokines. Further, arrays of MDDCs from alcohol and marijuana users confirm alcohol and cannabinoids inversely modulate inflammation. Oxidative stress results as measured by PCR array and DCFH-DA assay show MDDCs treated with EtOH produce higher levels of reactive oxygen species (ROS) and CBR agonists have the ability to modulate oxidative stress related genes and reduce the production of ROS. Overall, our results provide insights regarding the regulatory role CB2 and GPR55 agonists play in alcohol-induced inflammation. Supported by National Institute on Alcohol Abuse and Alcoholism, award K99AA021264.

Adjuvant effect of Imiquimod in genetic immunization with pHIV-gag

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Background & Objectives: There are around 34 million individuals living with HIV/AIDS worldwide. Although HAART has been effective controlling the disease, it has shown to be unsuccessful clearing the virus from infected people. Several groups have shown that Gag induces an HIV-specific T-cell immune response. We hypothesize that the adjuvant effect of the Imiquimod (I) will enhance the immune response of a DNA vaccine coding for HIV-Gag in a mouse model, after intramuscular immunization. Methods: We tested our hypothesis analyzing the cellular-immune response by measuring the IFN- γ production of mouse splenocytes by ELISPOT, and the TH1 and TH2 cytokine profiles by protein microarray. Results: Our data indicate that the mean frequency of the spot-forming cells (SFC) per million splenocytes was 12 ± 4 , 15 ± 4 , 55 ± 16 , 216 ± 98 , 209 ± 82 , 140 ± 40 , corresponding to naïve, pVax1, pGAG, pGAG+ I25, pGAG+ I50, pGAG+ I100, respectively. Our cytokine profile analysis shows mice immunized with pGAG+I25 to produce 804 pg/mL of IFN- γ . Conclusion: The proposed vaccination cocktail is augmenting the HIV-GAG vaccine-mediated production of IFN- γ on mouse spleens, and increases the IFN- γ in serum after vaccination. Our DNA-vaccine formulated with Imiquimod induces a TH1 cytokine milieu, amplifying the antigen-specific activation of cytotoxic-T lymphocytes, which is important against viral infections.

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Mechanisms involved in the HIV-1 induced amyloid beta nuclear uptake

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Clinical evidence indicates increased amyloid deposition in HIV-1-infected brains, which contributes to neurocognitive dysfunction in infected patients. Here we show that HIV-1 exposure stimulates amyloid beta nuclear entry in human brain endothelial cells (HBMEC), the main component of the blood-brain barrier (BBB). Treatment with HIV-1 and/or amyloid beta resulted in concurrent increase in early endosomal antigen-1 (EEA1), Smad, and phosphorylated Smad (pSmad) in nuclear fraction of HBMEC. A series of inhibition and silencing studies indicated that Smad and EEA1 closely interact by influencing their own nuclear entry; the effect that was attenuated by dynasore, a blocker of GTP-ase activity of dynamin. Importantly, inhibition of dynamin, EEA1, or TGF-beta/Smad effectively attenuated HIV-1-induced amyloid beta accumulation in the nuclei of HBMEC. The present study indicates that nuclear uptake of amyloid beta involves the dynamin-dependent EEA1 and TGF-beta/Smad signaling pathways. These results identify potential novel targets to protect against HIV-1-associated dysregulation of amyloid processes at the BBB level. Supported by MH063022, MH098891, NS39254, DA027569 and the Miami CFAR grant P30AI073961

Chronic morphine induced gut microbiome dysbiosis contributes to gut barrier dysfunction and systemic inflammation, which is exacerbated in HIV.

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Clinical observations show a strong correlation between microbial translocation and HIV disease progression. Chronic morphine, on its own, is known to induce bacterial translocation, impair immune activation and pathogenic clearance during infection and results in long-term persistent inflammation in the affected organs. Lack of response to therapy, and non-AIDS related comorbidities have been linked to immune activation as a consequence of microbial translocation. The gut microbiota plays a significant role in maintaining gut homeostasis and barrier integrity. Loss of microbes, which are part of the normal flora in healthy hosts, increase the susceptibility to a more virulent composition, that can contribute to barrier disruption and microbial translocation. In this study, we show that morphine induces bacterial translocation across gut mucosa, which is exacerbated with HIV infection. We also show HIV and morphine mediated gut microbiome changes and its influence on the immune system leading to persistent inflammation. Finally, we show that these co-morbidities preferentially favor class firmicutes over others and also increase adherence and alter virulence factors to tweak the host immune system to bring about the said effects. Supported by NIH grants 1R01DA034582, R01DA031202 and R01DA012104

Dopamine mediated activation of the ADAM17 metalloprotease in CD14+CD16+ monocytes: A mechanism for increased monocyte transmigration across the BBB in HIV infected drug abusers

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CD14+CD16+ monocyte transmigration across the blood brain barrier (BBB) is proposed to mediate HIV entry into the CNS and to contribute to the chronic neuroinflammation characteristic of HIV associated neurocognitive disorders (HAND). Studies suggest that substance abuse exacerbates HAND. We propose that increased extracellular dopamine in the CNS of HIV infected drug abusers contributes to CD14+CD16+ monocyte influx into the CNS, resulting in increased cognitive impairment. Using our model of the human BBB, we demonstrated that CD14+CD16+ monocytes obtained from uninfected and HIV infected individuals transmigrate across the BBB in response to dopamine. The D1-like dopamine receptor agonist SKF 38393 increased CD14+CD16+ monocyte transmigration across the BBB, indicating a role for the dopamine receptors D1DR and/or D5DR in the transmigration of this monocyte subpopulation. Although dopamine does not cross the BBB, activation of monocyte dopamine receptors once the cells have penetrated the BBB in response to low level chemokine production by endothelial cells and/or astrocytes may increase monocyte transmigration. The metalloprotease, a disintegrin and metalloprotease 17 (ADAM17), is involved in monocyte transmigration across vascular endothelium and we

demonstrated that dopamine increases expression of the active form of ADAM17, and perhaps MMP-9, by CD14+CD16+ monocytes. Dopamine mediated activation of ADAM17 and MMP-9 may therefore increase CD14+CD16+ monocyte transmigration across the BBB, contributing to the severity of HAND in HIV infected drug abusers. Supported by NIDA DA025567; NIMH MH075679 and MH090958

Impact of substance abuse on HIV-1-associated neurological decline in the DREXELMED HIV/AIDS Genetic Analysis Cohort

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The DREXELMED HIV/AIDS Genetic Analysis Cohort in Philadelphia, PA follows over 500 HIV-1-infected individuals longitudinally to examine viral genetic variation in conjunction with clinical and neurological disease severity and the impact of comorbidities like drugs of abuse on these parameters. This substantial cohort allows for a unique analysis of neurological decline in the context of substance abuse. Along with standard clinical parameters such as CD4+/CD8+ T-cell count, viral load measurements, and drug testing, a modified version of the Hopkin's Dementia Bedside Test (TMHDS) was performed. Due to the longitudinal nature of the DREXELMED cohort, it has been possible to investigate the complex effects of drug abuse on HIV-1-infected individuals. The complex nature of drug abuse complicates the traditional method of grouping patients into mono-use categories. Instead traditional methods were abandoned in favor of Markov Chains. These chains model patients as 'stateful machines' in which the change in HIVD score at the next visit is a function of parameters measured at the current visit. The current viral load, CD4+/CD8+ T-cell counts, adherence to HAART therapy, and drug testing results were included in the model. Cocaine use was found to have a detrimental effect on the TMHDS score while cannabinoids exhibited a small but seemingly measurable protective effect. As this study progresses, we will work to develop neurological testing protocols with increased sensitivity that will allow us to better understand the effects of poly-abuse on neurocognitive decline. Supported by This work is supported by NIH/NINDS R01 NS32092, NIDA R01 DA19807, NIMH P30 MH092177, and NIMH T32 MH079785.

Cocaine enhances HIV-1 integration in CD4+ T cells by modulating the epigenetic DNA signatures of host genome

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Cocaine, a commonly used drug among HIV-1 positive individuals, has been demonstrated to increase HIV-1 replication in permissive cells and humanized mouse. The goal of this study is to examine the effects of cocaine on HIV-1 integration-a critical step in HIV-1 life cycle. Our data demonstrates that cocaine treatment enhances HIV-1 integration in primary CD4+ T Cells. Our DNA methylation analysis reveals that cocaine induces global DNA demethylation in primary CD4+ T cells. Interestingly, DNA demethylating agent (5-aza-2'-deoxycytidine) also increased HIV-1 integration in CD4+ T cells. Given that integration has been suggested to be disfavored in methylated DNA targets, we hypothesize that cocaine enhances HIV-1 integration by reducing the DNA methylation signature of the host genome. Our in vitro studies with isolated PICs from infected cells illustrate that integration is inhibited in methylated target DNA. Furthermore, our in vitro integration assay strongly suggests that cocaine enhances HIV-1 integration. To best of our knowledge this is the first time a direct role of cocaine on HIV-1 integration has been demonstrated. Therefore, we believe understanding the mechanism by which cocaine modulates HIV-1 integration will aid in the discovery of novel targets that can synergize with integrase inhibitors as part of an approach to cure HIV/AIDS. Supported by T32 grant #2T32H007735-17 from NIH/NHLBI to Dr. S. E. Adunyah

Modulation of mir-146a expression by sumoylation in astrocytes

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Neuroinflammation plays a critical role in the pathogenesis of numerous neurodegenerative disorders including NeuroAIDS. Release of pro-inflammatory cytokines such as interleukin (IL)-1 β from infiltrating macrophages, activated microglia, astrocytes, and dysregulation of inflammation-associated microRNAs such as miR-146a within the brain can accelerate the development of HIV Associated Neurocognitive Disorder. We have demonstrated earlier that IL-1 β induces mir-146a synthesis in astrocytes in a C/EBP dependent manner. We now investigated whether C/EBP mediated mir-146a promoter activation is regulated by sumoylation, as part of the epigenetic regulation of transcription. Our studies demonstrate that overexpression of SUMO-1 mutant induces mir-146a expression while E3 SUMO ligase, PIASy inhibits both C/EBP-b isoform LAP and C/EBP-d mediated mir-146a promoter activation. On the contrary, overexpression of SUMO protease, SENP1 further enhances the transcriptional response of the mir-146a by C/EBP isoforms. Furthermore, site directed mutagenesis of the SUMO recognition motif ψ KXE/D in LAP and C/EBP-d increases mir-146a promoter activity. Our studies demonstrate that modification of the transcription factor C/EBP by sumoylation and desumoylation play significant role in mir-146a gene regulation. These observations highlight the potential of the SUMO pathway as a putative drug target in neurodegenerative diseases. Supported by NIH/NIDA

Development of hybrid nanocarrier (Au/Fe₃O₄) for active drug delivery to brain to eliminate HIV-1 persistence

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Development nanomaterials with less toxic and more biocompatible are ideal for drug targeting and delivery to brain by crossing blood-brain barrier (BBB). For the first time a hybrid carrier containing Au/Fe₃O₄ as shell and core was developed in our study. Inner Fe₃O₄ core maintains all the property of iron oxide and its movement can be directed by external magnetic force. Meanwhile, outer gold layer on the surface can provide not only high biocompatibility, but also higher quantity in drug binding and sustained release. TEM and DLS results showed that hybrid size was ~20 nm. XRD assay confirmed the element analysis of Au and Fe₃O₄ were respectively 22.3 % to 77.7 %. Classical vibrating sample magnetometer experiment showed that Au layer coating did not affect the superparamagnetism of Fe₃O₄. With maximum drug loading efficiency on NPs (93.2 $\pm$ 2.2% for Abacavir and 91.2 $\pm$ 3.2% for Lamivudine, respectively), Au/Fe₃O₄ exhibited high biocompatibility from hemolysis and MTT cytotoxic assays. Drug release profile of Abacavir from NPs showed a typical sustained release property at pH 6.0 and 7.4. In vitro BBB transmigration study, drug-Au/Fe₃O₄ demonstrated significant HIV-1 inhibition on HIV-1 infected primary human astrocytes by p24 assay. In vivo fluorescent images confirmed significant transmigration of liposomal Au/Fe₃O₄ nanoformulation in Balb/C mice brain under 0.2Tesla magnetic force. H&E pathology on main organs manifested negligible toxicity of NPs to mice. We suggest that the hybrid carrier may better serve as biocompatible nanocarriers to deliver the drugs to brain. Supported by NIH/1R01DA027049, R21MH101025, R01DA034547

Enhanced nicotine metabolism in HIV-1-positive smokers compared to HIV-negative smokers: Simultaneous determination of nicotine and its metabolites in their plasma using a simple and sensitive ESI-LC-MS/MS technique

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Smoking is approximately three times more prevalent in HIV-1+ than HIV- individuals in the USA. Nicotine, which is the major constituent of tobacco, is rapidly metabolized mainly by cytochrome P450 (CYP2A6) to many metabolites. In this study, we developed a simple and sensitive ESI-LC-MS/MS method using solid phase extraction, and determined the concentration of nicotine and its four major metabolites (cotinine, nornicotine, norcotinine, and trans 3'-hydroxycotinine) in the plasma of HIV-1-positive and HIV-negative smokers. The multiple reaction monitoring transitions for nicotine, cotinine, trans 3'-hydroxycotinine, nornicotine, norcotinine, nicotine-d4 and cotinine-d3 were selected at m/z 163.3/117.1, 177.5/80.3, 193.2/80.1, 149.5/132.3, 163.4/80.3, 167.3/121.4 and 180.3/101.2, respectively. The lower limit of quantitation for nicotine and its metabolites were 0.53 ng/mL, showing that this method is relatively more sensitive than those previously reported. The level of nicotine was detected 5-fold lower in HIV-1-positive smokers (7.17 $\pm$ 3.8 ng/mL) than that observed in HIV-negative smokers (33.29 $\pm$ 15.4 ng/mL), while the level of the metabolite nornicotine was 3-fold higher in HIV-1-positive smokers (6.8 $\pm$ 2.9 ng/mL) than HIV-negative smokers (2.3 $\pm$ 1.2 ng/mL). Although statistically non-significant, the level of the metabolite cotinine was also higher in HIV-1-positive smokers (85.6 $\pm$ 60.5 ng/mL) than those HIV-negative (74.9

±40.5 ng/mL). In conclusion, results support the hypothesis that nicotine metabolism is enhanced in HIV-1-positive smokers compared with HIV-negative smokers. Supported by NIH-DA031616-01

A novel validated and sensitive LC-MS/MS method in monkey plasma for simultaneous determination of methamphetamine and its metabolites in methamphetamine-treated monkey plasma

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Methamphetamine (MA), which is highly prevalent in HIV+ population, has been shown to increase HIV-1 pathogenesis through mechanisms including oxidative stress. We propose that MA metabolism is responsible for MA-mediated HIV-1 pathogenesis via increased oxidative stress. To test the hypothesis, we first developed and validated an electrospray ionization LC-MS/MS multiple reaction monitoring (MRM) technique in monkey plasma for determination of MA and its metabolites concentration. The MRM transitions for MA, 4-OH-MA, amphetamine (AM), 4-OH-amphetamine (4-OH-AM), and norephedrine were optimized with proton adducts [M+H]⁺ at m/z 150.5/91.2, 166.3/135.4, 136.4/91.3, 152.3/135.2, and 152.3/134.4, respectively. These analytes were extracted from monkey plasma using solid phase (SPE) extraction. The sensitivity of LC-MS/MS method was optimized at 1.0 ng/mL as a lower limit of quantitation. The method was linear over the range of 1-800 ng/mL (r²=0.9987-0.9996, accuracy≤9.8%). Further, the analysis of MA and its metabolites in MA-treated monkey plasma showed ~10-fold higher concentrations of AM (27 ng/mL) and 4-OH-AM (28 ng/mL) than MA (2.6 ng/mL), suggesting that MA is rapidly metabolized and the metabolites have relatively long half-life. In conclusion, we validated a novel and sensitive LC-MS/MS method using SPE for simultaneous analysis of these analytes in monkey plasma, which can be further applicable for their bioanalysis. We are now in the process of determining the relative levels of MA and its metabolites in HIV-infected and uninfected monkeys. Supported by NIH- DA025528 and DA025011

Reduction of Excessive Drinking in Alcohol-Dependent Rats via Soluble Epoxide Hydrolase Inhibition

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Alcoholism (or alcohol dependence) is a chronic relapsing disorder characterized by compulsive drinking and a dysregulation of affective states that may promote an acceleration of drinking in the context of negative reinforcement. The development of neuropathic pain in human alcoholics may engender such emotional distress and promote drinking. Hyperalgesia is also observed in animal models of dependence as nociceptive thresholds are lowered following excessive (but not limited) alcohol exposure, although the exact mechanisms linking pain and the motivation to drink are unknown. Epoxyeicosatrienoic acids (EETs) are lipid mediators that exhibit analgesic and anti-inflammatory properties. As EETs are inactivated via soluble epoxide hydrolase (sEH), inhibition of sEH is considered a valid therapeutic strategy targeting a variety of inflammatory disease states affecting numerous physiological systems. Interestingly, sEH is expressed throughout the brain, primarily in astrocytes, although the enzyme is also enriched in neurons of the central amygdala, a region hypothesized to mediate both the affective dimension of pain as well as alcohol dependence. In the current study, we found that systemic sEH inhibition (sEHI 1728, 0.3-1.0 mg/kg) reduced excessive alcohol self-administration in animals made dependent via chronic intermittent alcohol vapor exposure. Importantly, sEH inhibition did not affect drinking in non-dependent animals or water intake. These data suggest that systemic elevation of EETs may prove beneficial in reducing the compulsive drinking observed in alcoholics. Supported by AA020839 (SE) AR062866 (BI, BDH) AA008459 (GFK)

Opiate and encephalitis mediated dysregulation of autophagy in HIV infected microglia

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Here we have examined the role of autophagy as a potential mechanism by which Human Immunodeficiency Virus (HIV) type-1 infected microglia mediate neurotoxicity and determined whether opiates converge at these points. In addition we examined the role of autophagy in HIV-1-associated encephalitis, the pathologic hallmark of neuroAIDS. We showed that (i) inhibition of the autophagy pathway with 3-methyladenine (3-MA) and RNA interference against the autophagic protein, Beclin1, in HIV-1SF162 infected human microglia caused a decrease in viral titers by p24 ELISA, while co-exposure with morphine caused an increase in viral production despite the silencing of Beclin1; (ii) inhibition of autophagy caused a decrease in HIV-1 $\pm$ morphine-induced release of MCP-1, RANTES, IL-6 and TNF- α ; and (iii) human brain tissues from HIV-1-infected patients with varying levels of neurocognitive impairment $\pm$ HIV encephalitis (HIVE), many of which had a known history of substance abuse, showed qualitative differences in the autophagic proteins Beclin1, Autophagy-related gene (Atg)-7, and LC3-II by immunohistochemistry, and significant quantitative increases in Atg7 and p62/SQSTM1 mRNA levels from HIVE positive individuals using qRT-PCR. Overall these novel findings provide new insights into the underlying mechanisms of the enhanced HIV-1 pathogenesis in microglia in an opioid abusing population, and suggest that dysregulation of autophagy has significant involvement in encephalitis and opiate mediated neuropathogenesis related to HIV infection. Supported by NIH DA036154

Matrix metalloproteinases mediate intestinal epithelial tight junction disruption by quorum sensing molecule N-3-(oxododecanoyl)-homoserine lactone

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The intestinal epithelium forms a selective barrier maintained by tight junctions (TJs) and separating the luminal environment from the submucosal tissues. N-acylhomoserine lactone (AHL) quorum sensing molecules produced by Gram-negative bacteria in the gut can influence homeostasis of the host intestinal epithelium. In this study, we evaluated the regulatory mechanisms affecting the impact of two representative AHLs, N-3-(oxododecanoyl)-homoserine lactone (C12-HSL) and N-butyryl homoserine lactone (C4-HSL), on barrier function of human intestinal epithelial Caco-2 cells. Treatment with C12-HSL, but not with C4-HSL, perturbed Caco-2 barrier function; the effect that was associated with decreased levels of TJ proteins, occludin and tricellulin, and their delocalization from the TJs. C12-HSL induced also matrix metalloproteinase (MMP)-2 and MMP-3 activation through lipid raft- and protease-activated receptor (PAR)-dependent signaling. Pretreatment with lipid raft disruptors, PAR antagonists, or MMP inhibitors restored the C12-HSL-induced loss of the TJ proteins and increased permeability of Caco-2 cell monolayers. These results indicate that PAR/lipid raft-dependent MMP-2 and -3 activation is required for C12-HSL-induced alteration of epithelial paracellular barrier function through degradation of occludin and tricellulin in intestinal epithelial cells. Supported by American Heart Association 09SDG2300037 (SY Eum), NIH awards, P42 ES 07380, CA133257, DA027569, MH072567, MH098891 (M. Toborek)

Methamphetamine Alters Microglia Immune Function Through P2X7 Signaling

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Methamphetamine (METH) is a highly abused psychostimulant that is associated with neuroinflammatory and neurodegenerative pathology. Microglia, the endogenous macrophages of the central nervous system, respond to neurotoxic insults ensuing METH abuse. Recent literature implicates METH in microglial activation and neuroinflammation. However, the mechanism by which METH modulates microglia function remains elusive. Purinoceptors have emerged as mediators of chronic inflammation and neurodegenerative processes. The ionotropic purinoceptor P2X7 (P2X7R) is known to modulate pro-inflammatory signaling and integrate neuronal-glial circuits. In this study, we investigated the effects of METH on microglia purinoceptors. Stimulation of cultured microglia with METH (50 μ M) for 24H significantly increased P2X7 mRNA. Additionally, our studies in an escalating dose METH animal model demonstrated increased P2X7R expression in striatum compared to controls. METH (50 μ M) increased microglial migration towards Fractalkine (CXCL3) by 70% following 48H of treatment compared to control ($p < 0.001$). P2X7R antagonist pre-treatment prior to METH significantly reduced METH-induced phagocytosis ($p < 0.001$). Our findings suggest that P2X7 plays an important role in METH-induced microglia I activation responses.

Neuron mitochondria dynamics during HIV infection: Exploits of HIV-tat and gp120 in neuronal mitophagy and mitochondria biogenesis

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Approximately 50% of HIV+ persons are afflicted by some variant of HIV-associated neurocognitive disorders (HAND). HIV enters the brain causing infection of resident CD4+ cells, viral replication, viral protein/toxin production and neuroinflammation. Some, or all, of these mechanisms culminate in neurodegeneration and HAND. We hypothesized that HIV proteins released from infected CNS cells enter bystander neurons and affect mitophagy and mitochondria biogenesis. To test this we assayed brain tissues from a well-characterized cohort of HIV- and HIV+ donors for expression of key mitophagy and mitochondria biogenesis proteins and complimented these studies with in vitro assays using HIV proteins and SH-SY5Y neuroblastoma cells. Mitochondrial fusion proteins mitofusin (mfn) 1 and 2 and Parkin were significantly decreased in brains of HIV+ donors, while mRNA levels were increased. Immunostaining showed altered mfn1/2 expression in neurons of HIV- versus HIV+ brains. HIV gp120 and tat cause differential changes in mfn 1/2, parkin and mitochondria electron transport chain (etc) protein expression in SH-SY5Y cells, accompanied by changes in mitochondria morphology. Interestingly, HIV tat decreased mitochondria genome copy number in SH-SY5Y cells, while gp120 caused an increase. Both tat and gp120 cause reduced ATP production. These data suggest HIV gp120 and tat may invade CNS neurons and subsequently disrupt mitochondria biogenesis leading to neurodegeneration and HAND. Interfering with this neurodegenerative mechanism may provide relief for HAND patients. Supported by National Institute of Neurological Disorders and Stroke

HIV-1 TAT INCREASES THE SENSITIVITY OF MORPHINE IN ENTERIC ILEUM NEURONS

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The gastrointestinal (GI) mucosa, controlled by the enteric nervous system (ENS), plays a major role in HIV enteropathy, a syndrome characterized by a chronic diarrheal illness in HIV-infected patients. HIV-1 Tat released from infected T-cells and macrophages is intrinsically enterotoxic and may disrupt enteric homeostasis through bystander effects in enterocytes and/or enteric neurons resulting in HIV-associated diarrhea. Opioids directly affect the ENS, causing severe constipation. Recent experimental studies in our lab have shown that Tat increases enteric neuronal excitability but little is known about the combined effects of opiate abuse and Tat on enteric neurons. In order to determine the effects of Tat (100 nM) in combination with morphine (0.3 μ M, 3 μ M) on the voltage dependence of steady-state activation/inactivation of sodium channels, we used a double-pulse protocol in which a variable conditioning pulse was applied from -100 mV to +50 mV in 10 mV for 50 ms followed by a test pulse. In Tat-treated neurons, the sensitivity to morphine was significantly enhanced. The V_{0.5} for steady-state inactivation was shifted in Tat-treated neurons at 0.3 μ M morphine with a 50% decrease in maximal availability at -100 mV. Similar effects were noted for 0.03 μ M morphine in Tat transgenic mice with a 40% decrease. Corresponding doses of morphine alone did not alter the maximal availability of sodium currents. Overall, data suggest that the sensitivity of enteric neurons to morphine is enhanced by HIV Tat, thus exacerbating the deleterious effects of morphine on GI motility. Supported by NIDA R01 DA018633, K02 DA027374, K99 DA033878, DK046367

ACTIVATION OF DOPAMINE RECEPTORS INCREASES HIV ENTRY INTO MACROPHAGES

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Macrophages are one of the principle cell types infected with HIV, and the primary source of virus in many tissues, including the CNS. Within the brain, macrophages are exposed to the neurotransmitter dopamine, which is elevated by using drugs such as cocaine and methamphetamine. We showed previously exposure to dopamine increases HIV replication in macrophages and alters their cytokine production. Our current data demonstrate that the dopamine mediated increase in HIV replication is due to an increase in the ability of HIV to enter macrophages. This effect occurs at dopamine concentrations above 10 nM and is not dose dependent. The entry pathway affected is CCR5 dependent, as antagonizing CCR5 with TAK779 completely blocks entry. However, the increased susceptibility to entry is not due to an increase in surface CCR5. Increased entry requires activation of DR as it is blocked by the pan-dopamine receptor antagonist flupenthixol. Both the D1-like and D2-like DR mediate the increased entry, suggesting the involvement of a common signaling pathway. These data suggest that while different drugs of abuse act through distinct pharmacologic mechanisms, dopamine mediates the addictive and reinforcing effects of many drugs of abuse and may be a common mechanism by which drugs of abuse contribute to HIV associated neuropathogenesis. Thus, in drug abusers and others with elevated CNS dopamine levels, the development of neuroinflammation and HAND may be accelerated by the effects of dopamine on macrophages. Supported by NIDA, NIMH

Role of de-acidification of endolysosomes in the neuronal effects of HIV-1 Tat

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HIV-1 Tat continues to be implicated as a causative agent of HIV-1 associated neurocognitive disorder (HAND). We found previously that HIV-1 Tat elevates endolysosome pH, alters the structure and function of neuronal endolysosomes, and increases amyloidogenesis in primary cultured neurons. De-acidification of endolysosomes may play a central role in these effects of HIV-1 Tat because activation of mucolipin transient receptor potential-1 cation channels (TRPM1-1) with ML-SA1 decreased significantly endolysosome pH as well as intra-neuronal levels of A β 1-40 and A β 1-42. Furthermore, HIV-1 Tat induced proton leakage out of endolysosomes may play an important role in the morphological and functional changes in endolysosomes because siRNA knockdown of the proton-coupled oligopeptide transporters blocked HIV-1 Tat-induced enlargement of endolysosomes. De-acidification of endolysosomes may participate further in the pathogenesis of HAND by affecting endolysosome calcium homeostasis. We have found that elevation of endolysosome pH not only induces calcium release from endolysosomes but also activates a novel endolysosome-dependent calcium influx mechanisms across plasma membranes, a phenomenon we have termed "acidic store-operated calcium entry". Thus, de-acidification of endolysosomes may play a central and early role in the pathogenesis of HAND and intervention against HIV-1 protein-induced morphological and functional changes to endolysosomes may become a new therapeutic strategy against HAND. (Supported by AG043338, GM103329) Supported by AG043338, GM103329.

AUTOCRINE ACTIONS OF SECRETED ANGIOTENSIN II AND GLUCOSE INTOLERANCE IN HUMAN NEURONAL CELLS

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The autocrine actions of the local Renin Angiotensin System (RAS) have been implicated in the etiology of insulin resistance in many tissues. However, the relationship between the brain secreted Angiotensin II (Ang II), the dominant effector of the RAS, and the benefits of Ang II Receptor Blockers (ARBs) in the neuronal glucose uptake (GU) has never been explored. In this study we overexpressed intracellular Ang II in cultured neuronal cells and then analyzed the GU effects of the secreted peptide in the presence or absence of losartan. Neuronal SH-SY5Y cells were transfected with a plasmid containing the entire fragment of the Ang II gene (24bp) under the transcriptional regulation of the pCMV promoter using the Eugene Transfection Reagent. The levels of intracellular and secreted Ang II were quantified using a specific Ang II-EIA ELISA kit. GU was assessed using a fluorescent glucose (2-NBDG) and flow cytometry (FC). The effects of losartan (10⁻⁷M) in the GU by neuronal cells were analyzed after 24 hours in Ang II-transfected and untransfected cells. Our results indicated that: (1) Neuronal cells overexpressing Ang II significantly increased ($p < 0.05$) the levels of the secreted peptide to the culture medium, (2) GU significantly decreased ($p < 0.05$) in Ang II-transfected cells and this effect was antagonized by losartan; (3) Losartan was unable to change the GU in absence of secreted Ang II. Our studies demonstrate that the autocrine actions of Ang II in neuronal cells can play an important role in the pathophysiology of insulin resistance in the brain. Supported by R21MH095524, S11NS046278, U54NS043011, U54RR026139, U54MD007587, G12RR003051, G12MD007600, R25MH080661

Mechanistic evaluation of efavirenz neurotoxicity: Implications for cognitive impairment

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Efavirenz (EFV) is among the most commonly used antiretroviral (ARV) drugs globally, causes neurological symptoms that interfere with adherence and reduce tolerability, and may have CNS toxicity that might help to explain the high prevalence of HAND in patients on HAART (~ 50%). However, the mechanism of its CNS toxicity is unknown. We evaluated the EFV containing regimen: EFV (10 μ M)/zidovudine ([AZT] 10 μ M)/lamivudine ([3TC]

10 μ M) in murine N2a cells transfected with the human "Swedish" mutant form of APP (SweN2a cells). Those treated with EFV or EFV/3TC/AZT generated significantly increased soluble A β , and had increased β -secretase-1 (BACE1) expression while 3TC or AZT did not significantly alter these endpoints. We next tested these medications in amyloid-beta (A β) producing Tg2576 mice combined or singly using clinically relevant doses. EFV or EFV/3TC/AZT promoted significantly more BACE1 expression and soluble A β generation but 3TC or AZT did not. Further, EFV or EFV/3TC/AZT promoted significantly more mitochondrial stress (ROS production, ATP depletion, and disruption of mitochondrial membrane potential) in SweN2A cells as compared to 3TC, AZT, or vehicle control. Finally, microglial A β phagocytosis was significantly reduced by EFV or EFV/3TC/AZT but not by AZT or 3TC. These data suggest the majority of neurotoxicity of the 3TC/AZT/EFV regimen is dependent upon EFV as it promotes both increased production, and decreased clearance of A β peptide, two modes of neurotoxicity known to be promoted by mitochondrial stress. Supported by NIMH/NIH grant (1R01MH098737-02) (BG).

Cocaine mediated induction of microglial autophagy involves ER stress

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The role of HIV proteins in interfering with clearance pathways such as autophagy, has been well documented. Whether cocaine exposure of microglial cells leading to cellular stress involved induction of the autophagic pathway remains unexplored. Herein we demonstrate that cocaine exposure induced autophagosome formation both in BV2 as well as primary rat microglial cells as demonstrated by a dose- and time-dependent induction of autophagy signature proteins such as Beclin1, ATG5, and LC3-II in western blots. These findings were further validated by immunostaining wherein cocaine treatment of BV2 cells resulted in increased formation of puncta in cells expressing either endogenous LC3 or overexpressing GFP-LC3. Specificity of cocaine-induced autophagy was confirmed by treating cells with inhibitors of autophagy (3-MA & Wortmannin). Intriguingly, cocaine-mediated induction of autophagy involved upstream activation of two ER stress (PERK & IRE1 α -dependent) pathways, as evidenced by the ability of ER stress inhibitor, salubrins, to ameliorate cocaine-induced autophagy. In vivo validation of these findings demonstrated increased expression of Beclin1, ATG5, & LC3-II protein expression as evidenced by western blotting and immunostaining in cocaine-treated mouse brains compared to untreated animals. Increased autophagy was linked to cocaine-mediated activation of microglia. In summary, our findings identify the role of ER stress/autophagy pathway in cocaine-mediated activation of microglia. Supported by NIH grants: DA020392, DA023397, and DA024442

Anti-inflammatory and neuroprotective properties of insulin in preclinical models of HIV-Associated Neurocognitive Disorders

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Despite cART a state of sustained systemic and CNS inflammation exists, and contributes to HIV-Associated Neurocognitive Disorders (HAND). Glial activation and neuronal injury are hallmarks of CNS damage in HIV. The ideal therapeutic would both protect neurons and dampen inflammation. Insulin has neurotrophic, neuromodulatory, and anti-inflammatory effects. We found that insulin protected primary hippocampal neurons from HIV-protein (X4, R5 and dual gp120), inflammatory (TNF α , IL-1 β) and excitotoxic (NMDA) insults in a concentration related manner. Using a mitochondrial stress test, we found that insulin protected mitochondrial respiration. As respiration is largely dependent on substrate delivery to mitochondria, while proton efflux is considered to be an ATP-independent mechanism that regulates membrane potential, these data suggest insulin preserved the delivery of energy substrates to mitochondria in a manner independent of alterations in electron transport. To determine if insulin dampens microglial activation we measured the effect of insulin on extracellular glutamate and TNF α levels in rat primary microglia treated with lipopolysaccharide or Tat protein. We found that insulin dose dependently reduced glutamate and TNF α release from microglia. We next conducted intra-cerebral injections of Tat +/- insulin in mouse brain. Tat injection shifted resting microglia to an activated phenotype, and promoted the influx of leukocytes (neutrophils). Insulin dose dependently prevented both microglial activation and leukocyte influx. These preliminary findings suggest that insulin delivered to brain can reduce neuroinflammation by suppressing microglial activation, and protects neurons by preserving cellular energetics. The direct delivery of insulin to brain (i.e. intranasal) is a promising strategy to protect the

Cannabinoids and Immunosuppression: MicroRNA expression profiling and target analysis in the induction of myeloid-derived suppressor cells (MDSC) by THC in vivo reveals regulation of C/EBP α by miR-690

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Delta-9-tetrahydrocannabinol from marijuana has been shown to induce potent MDSC in vivo. We performed transcriptional profiling of miRNA in CD11b+Gr-1+ MDSC induced by THC. MDSC were purified from peritonea of BL/6 mice administered with 20 mg/kg THC. Purified CD11b+Gr-1+ MDSC precursors from BM of naive mice served as controls. Genome-wide microarray analysis of 690 mouse miRs showed markedly altered expression profile in THC-MDSC vs control BM precursors. We validated the results by real-time qPCR and identified 13 differentially expressed miRNA (>2 fold). A combination of TargetScan, RNAhybrid and miRanda algorithms were used to identify the putative targets (Poisson P <0.05; predicted by at least 2). Ingenuity pathway analysis of targets showed cell growth, proliferation, and myeloid differentiation as the top significantly enriched canonical pathways associated with THC-MDSC. MiR-690, overexpressed in THC-MDSC (~16 fold), was predicted to target mRNA 3'UTR of transcription factor C/EBP α involved in myeloid differentiation. Consequently, C/EBP α mRNA and protein levels were higher in BM precursors with a significant decrease in THC-MDSC. Knock-down of miR-690 in THC-MDSC using transfection with peptide nucleic acid antagomiR resulted in significant increase in the expression of C/EBP α . Analysis of miR-690 and C/EBP α in purified granulocytic and monocytic MDSC subtypes suggested that miR-690 is a pan-MDSC regulator. In conclusion, select miRNA and their targets may mediate the induction of MDSC following exposure to cannabinoids in vivo resulting in immune suppression. Supported by NIH P01AT003961, P20RR032684, R01AT006888, R01ES019313, R01MH094755 & VA 1J01BX001357

Infiltrating regulatory B (Breg) cells control neuroinflammation following viral brain infection

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In vivo and in vitro experiments were undertaken to elucidate the role of regulatory B lymphocytes in controlling neuroinflammation following brain infection with murine cytomegalovirus (MCMV). This unique subset of CD19(+)CD1d(hi)CD5(+) B-cells was found to infiltrate the brains of chronically infected animals, reaching highest levels at the latest time point tested (35 d p.i.). B-cell-deficient Jh-/- mice (BKO) displayed exacerbated neuroimmune responses when compared to infected, wild-type (Wt) animals as measured by enhanced accumulation and/or retention of CD8+ T lymphocytes within the brain, as well as increased levels of microglial cell activation (MHC class II). Conversely, levels of regulatory T-cells (Tregs) were found to be significantly lower in infected BKO mice when compared to Wt animals. Further experiments showed that in vitro generated interleukin (IL)-10-secreting Breg cells were able to inhibit cytokine and chemokine responses from microglia following stimulation with viral antigens. In addition, these in vitro generated Bregs were also found to promote conversion of CD4+ T-cells into a regulatory T-cell (Foxp3+) phenotype. Finally, gain-of-function experiments demonstrated that reconstitution of B-cells into BKO mice restored these neuroimmune responses to levels exhibited by infected Wt animals. Taken together, these results demonstrate that regulatory B-cells modulate T lymphocyte as well as microglial cell responses within the brain, and promote CD4+ T-cell transition into a Treg phenotype. Supported by NINDS/NIH RO1 NS038836

Microvesicles mediate glutaminase release and excess glutamate production: Implication for HIV-1 associated neurocognitive disorder

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HIV-1-infected and/or activated microglia and macrophages are pivotal in the pathogenesis of HIV-1-associated neurocognitive disorders (HAND). These cells produce a range of neurotoxic factors, including excess levels of glutamate. Glutaminase, a mitochondrial enzyme that produces glutamate, is upregulated in HAND. However, the mechanisms by which glutaminase uses to elevate glutamate remain unclear. Recent evidence suggests

microvesicles (MVs), which contain enzymes, RNAs, cytokines and chemokines, are released into the brain microenvironment. We hypothesized that MV release of glutaminase is critical in HIV-1 infection leading to neurotoxicity. We demonstrated that HIV-1-infected human primary microglia and monocyte-derived macrophages had higher levels of MVs, which were isolated through differential centrifugation. The elevated MV was confirmed by Western blotting and immunocytochemistry using antibodies against MV-specific antigens. To further determine the MV functions, we used reverse phase high-performance liquid chromatography to quantify the excess glutamate generation by MVs. More importantly, blocking glutaminase activity with the glutaminase inhibitors in MVs dramatically reduced the glutamate levels, suggesting HIV-1-infected microglia and macrophages release glutaminase into extracellular fluid through MVs. To conclude, MVs pathway is a potential mechanism for glutaminase release and excess glutamate generation in HAND. Understanding the pathway could help to identify novel therapeutic targets for neuronal protection in HAND. Supported by R01 NS 41858-01 and R01 NS 061642-01

Toll-like receptor 3 signaling in gut epithelial cells activates type III IFN pathway

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Toll-like receptor 3 (TLR-3) recognizes double stranded RNA and induces multiple intracellular events responsible for innate antiviral immunity against a number of viruses, including HIV-1. However, there is limited information about intestinal epithelia cells in gut innate immunity against HIV-1. We thus examined the role of intestinal epithelia cells in activation of antiviral factors. We demonstrated that human intestinal epithelia cells (HCT116) expressed functional TLR-3 that could be activated by PolyI:C, resulting in the induction of endogenous type III IFNs (IFN-lambda), surprisingly not IFN-alpha/beta. TLR-3 signaling of epithelial cells also induced IFN-stimulating genes (ISGs), including OAS-1 and RANTES. In addition, TLR-3 activation increased the expression of RIG-I, an intracellular sensor of viral replication. Furthermore, Epigallocatechingallate (EGCG), a known anti-inflammatory molecule found in green tea, could suppress TLR-3 signaling-mediated upregulation of IL-8, RANTES and OAS-1 in the epithelia cells. These findings indicate that intestinal epithelia cells participate in the gut innate immunity producing the intracellular antiviral factor. Future studies are necessary in order to determine if the antiviral factors (particularly IFN-lambda) released by intestinal epithelia cells have the ability to inhibit HIV infection/replication. Supported by NIH DA36163, DA22177, DA27550

Lectin-based passive and active dendritic cell immunotherapies against neuroinflammatory disorders

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The potential of dendritic cells (DCs) as sentinels for immunotherapy is emerging against various diseases such as HIV, multiple sclerosis (MS) and various cancers. During neuroinflammation, DCs transmigrate across the blood-brain barrier (BBB) into the CNS using adhesion receptors in the presence of chemoattractants, particularly CCL2, and initiate immune response. We have shown through near-infrared imaging that DC accumulation into lesions within the CNS correlated with severity of inflammation during experimental autoimmune encephalitis (EAE), a model for MS. An in vitro BBB model revealed that DCs migrated paracellularly and exhibited more efficient transmigration than T cells in the presence of CCL2. Different DC subsets- myeloid (mDC), plasmacytoid (pDC) and monocyte-derived (MDDC) had a unique enrichment of lectin receptors; each subset utilizing a different lectin receptor to adhere and transmigrate in the presence of CCL2. At the molecular level, a cross talk between actin and lectin receptor pathways was also established. While lectins can be selectively targeted on DCs to attenuate neuroinflammation, they can also be harnessed to enhance cytotoxic response against tumors. Glioblastoma multiforme (GBM), one of the most malignant types of brain tumor served as our model for the uptake of tumor antigens via specific lectin receptors on DCs. First, we reversed the effect of cancer-driven hypoxia on DC functionality and then showed potentiation of DC migration and antigen specificity using this glycotargeting approach. In conclusion, these studies will further substantiate the promise of DC-based immunotherapies by generating lectin-targeted therapies that can be directed to inflammatory lesions or tumors.

Effect of morphine and SIV on dendritic cell trafficking into the central nervous system of rhesus macaques

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Recruitment of immune cells such as monocytes/macrophages and dendritic cells (DCs) across the blood-brain barrier (BBB) has been documented in diseases involving neuroinflammation. Neuroinvasion by HIV leads to neurocognitive diseases and alters the permeability of the BBB. Likewise, many HIV patients use drugs of abuse such as morphine, which can further compromise the BBB. While the role of monocytes and macrophages in neuroAIDS is well established, research demonstrating the presence and role of DCs in the CNS during HIV infection has not been developed yet. In this respect, this study explored the presence of DCs in the brain parenchyma of rhesus macaques infected with a neurovirulent form of SIV (SIV mac239 R71/17E) and administered with morphine. Cells positive for DC markers including CD11c (integrin), macDC-SIGN (dendritic cell-specific ICAM-3 grabbing nonintegrin), CD83 (a maturation factor) and HLA-DR (MHC class II) were consistently found in the brain parenchyma of SIV-infected macaques as well as infected macaques on morphine in correlation with SIV viral loads in CSF, plasma, and frontal cortex. Control animals did not exhibit any DC presence in their brains. These results provide first evidence of DCs' relevance in NeuroAIDS vis-à-vis drugs of abuse and open new avenues of understanding and investigative HIV-CNS afflictions leading towards resolution of viral reservoirs within CNS compartments.

In vivo immunogenicity of Tax 11-19 epitope in HLA-A2/DTR transgenic mice: implication for dendritic cell-based anti-HTLV-1 vaccine

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Viral oncoprotein Tax plays key roles in transformation of human T-cell leukemia virus (HTLV-1)-infected T cells leading to adult T-cell leukemia (ATL), and is the key antigen recognized during HTLV-associated myelopathy (HAM). In HLA-A2+ asymptomatic carriers as well as ATL and HAM patients, Tax(11-19) epitope exhibit immunodominance. Here, we evaluate immunotherapeutic potential of this epitope in HLA-A2 transgenic mice in the presence and absence of dendritic cells (DCs) given the recent encouraging observations made with Phase 1 DC-based vaccine trial for ATL. To facilitate these studies, we first generated an HLA-A2/DTR hybrid mouse strain carrying the HLA-A2.1 and CD11c-DTR genes. We then studied CD8 T-cell immune response against Tax(11-19) epitope delivered in the absence or presence of Freund's adjuvant and/or DCs. Overall results demonstrate that naturally presented Tax epitope could initiate an antigen-specific CD8 T cell response in vivo but failed to do so upon DC depletion. Presence of adjuvant potentiated Tax(11-19)-specific response. Elevated serum IL-6 levels coincided with depletion of DCs whereas decreased TGF- β was associated with adjuvant use. Thus, Tax(11-19) epitope is a potential candidate for the DC-based anti-HTLV-1 vaccine and the newly hybrid mouse strain could be used for investigating DC involvement in human class-I-restricted immune responses.

Irradiation-induced cellular stress activates virus replication and apoptosis of HIV-1 infected cells in vitro and in vivo

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Active antiretroviral therapy (HAART) reduces HIV RNA in plasma; however, virus persists in resting T cells and other reservoirs: perivascular macrophages and microglia. X-ray irradiation (IR), a well-defined stress signal that is used for therapeutic purposes, including therapy of HIV-1 associated lymphoma, is shown to activate HIV-1 transcription, progeny virion formation, and apoptosis of infected cells. Using HIV-1 infected T-cells, monocyte-derived macrophage (MDM) model, and NSG humanized mice infected with dual-tropic HIV-1 89.6 strain, the effects of therapeutic IR doses on HIV-1 replication were examined. Exposition of both PBMCs and MDM to IR led to a dramatic increase of HIV-1 transcription, evidenced by presence of Pol II and the reduction of HDAC1 on the HIV-1 promoter. IR treatment of CD4+ T cells resulted in enhanced apoptosis of infected cells due to increased phosphorylation of S46 on p53 protein. Incubation of IR-treated cells with proteasomal inhibitor ALLN also results in an increase of HIV-1 transcription possibly due to Tat protein stabilization. The virus produced by irradiated cells reduces infectivity, suggesting IR damages virion assembly pathways or increases production of defective viral particles. Treatment of HIV-1 infected humanized mice with no viral RNA in the plasma or PBMCs with IR resulted in a drastic increase of HIV-1 RNA in plasma, lung and brain. Our data suggests that IR-induced cellular stress activates HIV-1 expression in viral reservoirs and facilitates the apoptotic death of infected cells via Tat-dependent p53 phosphorylation. Supported by NIH/NIAID

Macrophage-derived cysteine leukotriene's play a critical role in HIV-associated neuronal injury.

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Recently, we found that p38 MAPK signaling is essential for both the neurotoxic phenotype of HIV/gp120-stimulated macrophages, and the induction of neuronal apoptosis initiated by HIV/gp120-induced macrophage toxins. Our present study aimed to characterize the mechanistic link in macrophages between HIV co-receptors, p38 MAPK and events downstream of the protein kinase. Using genome-wide gene expression analysis, we compared macrophages stimulated for 4 or 24 hours with HIV/gp120SF162 (1 nM) and, as pro-inflammatory control, bacterial lipopolysaccharide (1 microM) in the presence and absence of the pharmacological p38 MAPK inhibitor SB203580 (10 microM). Follow-up experiments employed siRNA-based, biochemical and cell biological approaches. We confirmed that HIV-infection and gp120-stimulation significantly increased production of pro-inflammatory cysteinyl leukotrienes by macrophages. In contrast, inhibition or knockdown of p38 MAPK caused macrophages to produce multiple anti-viral and cytoprotective factors. Moreover, reduction of p38 MAPK activity down-regulated cysteinyl leukotriene synthase (LTC4S) in macrophages. Finally, we discovered that pharmacological inhibition of the cysteinyl leukotriene receptor 1 (CysLTR1) protected cerebrocortical neurons against toxicity of both gp120-stimulated and HIV-infected macrophages suggesting a critical role for macrophage-derived cysteinyl leukotrienes in HIV-associated neuronal injury. Supported by NIH grants R01 MH087332, DA026306 and DA029480 (to M.K.)

Impact of habitual marijuana (MJ) use on humoral and cellular immune responses to a hepatitis B virus (HBV) vaccine

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Exposure to δ^9 -tetrahydrocannabinol (THC) in vitro and in animal models has been shown to alter the differentiation, activation and function of dendritic cells, T cells and B cells. However, studies that directly address the impact of MJ smoking on functional human immune responses are lacking. We hypothesized that the potent immunosuppressive effects of THC would translate into a marked impact on the capacity for habitual MJ smokers to develop antigen-specific responses when vaccinated. A matched cohort of 9 MJ smokers (MS) and 9 nonsmokers (NS) were vaccinated with a standard 3-dose regimen of HBV vaccine (0, 1 and 6 months). MS were admitted to our Clinical Research Center on the day of each vaccination and smoked four MJ cigarettes (3.75-3.95% THC, NIDA) over the course of 24 hrs, with their HBV vaccine injected just prior to the 3rd MJ cigarette. Blood samples were collected prior to and following MJ smoking to determine THC concentrations and subjects kept a diary of ongoing MJ use. NS were vaccinated as outpatients without MJ exposure. Serial blood samples were collected from all subjects to evaluate the development of HBV-specific B and T cell responses over time. Interestingly, no differences were observed between MS and NS subjects with respect to peripheral blood cell counts or phenotype, anti-HBV antibody levels, or HBV-specific T cell proliferation or cytokine production. While a larger cohort may be required to rule out a small suppressive effect, habitual MJ smoking has no major impact on the development of systemic immunity to HBV vaccine. Supported by NIDA/NIH R01 DA003018

Ashwagandha (*Withania somnifera*) reverses HIV-1 induced neurodegeneration via attenuation of β -Amyloid plaques: Implications in HIV-Associated Neurocognitive Disorders (HAND)

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Despite the advent of HAART, HIV-1 Associated Neurocognitive Disorders (HAND) continue to exist in more than 50% of HIV-1 infected patients. Further, the epidemiology of HIV-1 infection is increasing in aging population coincided with increasing HAND. Previous studies have shown that β -amyloid plaque is increasingly accumulated in HIV-1 infected brain suggesting synergistic effects of HIV-1 infection and β -amyloid on the genesis of HAND in HIV-1 infected aging population. *Withania somnifera* (WS) also known as 'ashwagandha' is used widely in Ayurvedic medicine as a nerve tonic and memory enhancer. We have recently reported that WS significantly reversed the β -Amyloid (1 α €42)-induced neuronal plasticity in neuronal cells (SK-N-MC). In this report, we hypothesize that, WS could reverse β -amyloid and /or HIV-1 (Ba-L) induced neuropathogenesis in SK-N-MC cells.

Our results showed that HIV-1/ β -amyloid induced cytotoxic effects in SK-N-MC cells were reversed by WS as shown by trypan blue staining, cellular localization, MTT formazan exocytosis, tau-phosphorylation and the levels of acetylcholinesterase activity. Further, the confocal microscopic analysis showed decreased spine density, loss of spines and decreased dendrite diameter, total dendrite and spine area in HIV-1 infected and /or β -amyloid treated SK-N-MC cells compared to uninfected or untreated control cells. However, when ashwagandha was added to HIV-1 infected / β -amyloid treated SK-N-MC cells, these synaptic dysregulatory effects were significantly reversed suggesting the neuro-protective effect of WS against β -amyloid and HIV-1 induced neuropathogenesis. Further studies are being carried out to isolate the active pharmaceutical moiety of WS to attenuate HIV-1 / β -amyloid induced plaque formation and restore neurocognitive functions in HIV-1 infected aging populations. Supported by This study was supported by NIH grants: 1R01MH085259, 1R01DA034547 and 1R21MH101025

Cocaine Induces Nuclear Export and Degradation of Neuronal Retinoid X Receptor- γ via a TNF- α /JNK- Mediated Mechanism*

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Cocaine abuse represents an immense societal health and economic burden for which no treatment currently exists. Among the numerous intracellular signaling cascades impacted by exposure to cocaine, increased and aberrant production of pro-inflammatory cytokines in the CNS has been observed. Additionally, we have previously reported a decrease in retinoid-X-receptor-gamma (RXR- γ) in brains of mice chronically exposed to cocaine. Through obligate heterodimerization with a number of nuclear receptors, RXRs serve as master regulatory transcription factors which can potentiate or suppress expression of a wide spectrum of genes. Little is known about the regulation of RXR levels, but previous studies indicate cellular stressors such as cytokines negatively regulate levels of RXRs in vitro. To evaluate the mechanism underlying the cocaine-induced decreases in RXR- γ levels observed in vivo, we exposed neurons to cocaine in vitro and examined pathways which may contribute to disruption in RXR signaling, including activation of stress pathways by cytokine induction. In these studies, we provide the first evidence that cocaine exposure disrupts neuronal RXR- γ signaling in vitro by promoting its nuclear export and degradation. Furthermore, we demonstrate this effect may be mediated, at least in part, by cocaine-induced production of TNF- α and its downstream effector c-Jun-NH-terminal kinase (JNK). Findings from this study are therefore applicable to both cocaine abuse and to pathological conditions characterized by neuroinflammatory factors, such as neurodegenerative disease.

PINCH levels in the CSF of HIV patients correlate with CD4 count

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Several studies report associations between the PINCH (particularly interesting new cysteine histidine-rich) protein and HIV-associated CNS disease. PINCH is detected in the CSF of HIV patients and levels may be indicative of changes in disease status over time. PINCH binds hyperphosphorylated Tau (hpTau) in the brain and CSF, but little is known about the relevance of these interactions to HIV CNS disease. In this study, PINCH and hpTau levels were assessed in three separate CSF samples collected longitudinally from 20 HIV+ patients at intervals of approximately 1, and 6 months from the initial visit (baseline). Correlational analyses were conducted for CSF levels of PINCH and hpTau and other variables including plasma CD4+ T-cell count, plasma and CSF viral burden, CSF neopterin, white blood cell (WBC) count, and antiretroviral CNS penetration-effectiveness (CPE). Statistical analyses showed that the fold-change in CSF PINCH protein levels were significantly higher in patients with CD4 counts <200 cells/mm³ compared to those with CD4 counts Supported by NIMH

Epigallocatechin gallate protects systemic endotoxin challenges-induced neuroinflammation

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Traditional medicine is a potential source in the search for and development of dietary or complementary supplements to reduce the risk of neurological disorders. Lipopolysaccharide (LPS)-mediated systemic

inflammation plays a critical role in neurodegenerative diseases. In the present study, we investigated the protective effects of epigallocatechin gallate (EGCG) on LPS-mediated inflammatory response from both in vitro and in vivo studies. Our results showed that LPS not only exerted neurotoxicity through activation of macrophages to secrete inflammatory cytokines, but also had direct neurotoxic effect to primary human neuronal cells. LPS treatment increased the expression of neurotoxic cytokines and the production of reactive oxygen species (ROS) in human primary neurons. EGCG pretreatment of macrophages or neurons suppressed LPS-induced ROS production and expression of neurotoxic cytokines, which attenuated the neuronal aggregation and neuronal death. To confirm these in vitro findings, we further investigated the anti-inflammatory effect of EGCG in mice. Injection of LPS to mice induced inflammatory responses. EGCG, when given to mice, significantly attenuated LPS-induced inflammation. These data suggest that EGCG represents a promising herbal medicine for the treatment of inflammation-mediated neurodegenerative diseases. Supported by NIH DA012815, DA027550, DA022177

Spatial learning and motor deficits in glutaminase C overexpression transgenic mice

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Many neurodegenerative diseases including HIV associated neurocognitive disorders (HAND) are often associated with progressive central nervous system (CNS) atrophy, loss of structure or function of neurons, and neuronal death. Glutamate, a product of glutaminase 1 (gls1), is a main excitatory neurotransmitter able to cause brain damage when in excess levels. Extensive literature has documented the association between elevated glutamate level and neurodegenerative diseases. However, little is known whether glutaminase upregulation is sufficient to cause behavioral abnormalities and neuronal injury in vivo. To determine the pathogenic effect of gls1 in the CNS, we first created glutaminase c (GAC), one of the gls1 isoforms, transgenic mouse model. CDS of GAC was cloned into a CAG-loxp-GFP-stop-loxp-IRES-LacZ plasmid. The plasmid DNA was then microinjected into fertilized eggs to generate founder lines. By mating the founder lines with Nestin-cre mouse, we generated Nestin-GAC mouse that overexpressed GAC in all Nestin-derived CNS cells. The overexpression of GAC was confirmed by Western Blotting and immunohistochemistry. We observed that the GAC overexpression was predominantly located in the Hippocampus and cerebral cortex. Furthermore, through Morris water maze and Rotarod tests, we found that the Nestin-GAC mice had significant spatial learning and memory and motor impairments when compared with their control mice. Therefore, this gls1 transgenic mouse model could potentially serve as a novel model of neural impairment as seen in HAND and other neurological diseases. Supported by NIH

Cross Talk Between p66ShcA and p70S6K Determines Kidney Cell Injury in HIV-Associated Nephropathy

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The functions of p66ShcA are different than its family members. In response to oxidant and apoptogenic stimuli, p66ShcA translocates to mitochondria, where it generates Reactive Oxygen Species (ROS) by transferring electrons from cytochrome c to oxygen. On that account, cells deficient of p66ShcA are redox-resistant phenotype. We recently reported that p66Shc deficient (p66-DF) status reactivated redox-sensitive stress response program (RSSRP) both in podocytes and tubular cells in HIV milieu. Mammalian Target Of Rapamycin (mTOR) regulates ribosomal protein synthesis, cell growth, cell cycle progression, autophagy, and Unfolded Protein expression (UPR, endoplasmic reticulum stress) by targeting p70S6K. In mammalian cells, its inhibition promotes autophagy and reduce intracellular accumulation of mis-folded proteins and thus attenuates UPR. Recently, we reported the role of mTOR pathway in the development of proliferative phenotype both in vivo and in vitro. Moreover, rapamycin, an inhibitor of mTOR pathway provided protection against HIV induced renal cell injury both in vitro and in vivo. We suggest that cross talk between p66ShcA and p70S6K determines Kidney Cell Injury in HIV-associated Nephropathy. Supported by NIH

CHRONIC BINGE ALCOHOL (CBA) DIFFERENTIALLY MODULATES HIPPOCAMPAL GENE EXPRESSION CRITICAL FOR CELL GROWTH, DIFFERENTIATION, INFLAMMATION, AND GABA FUNCTION IN SIMIAN IMMUNODEFICIENCY VIRUS (SIV)-INFECTED RHESUS MACAQUES

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HIV-associated neurocognitive disorder (HAND) remains prevalent despite the advent of effective antiretroviral therapy. Alcohol use disorders (AUD) exacerbate neurocognitive dysfunction in HIV+ patients. Previous studies in SIV-infected macaques administered CBA have shown increased plasma viral load at set point, accentuated muscle wasting, and more rapid progression to end stage. Moreover, CBA unmasked behavioral deficits in SIV-infected macaques. The mechanisms involved in accentuated wasting include inflammation, oxidative stress, and attenuated anabolic signaling; however, the underlying mechanisms of neurocognitive alterations are not known. The aim of this study was to explore the CBA-mediated alterations in brain gene expression in SIV-infected macaques. Transcriptome of hippocampal samples obtained at necropsy (16 months post-SIV) from sucrose-treated (SUC/SIV, N=2) and CBA (CBA/SIV, N=2) macaques were analyzed using Illumina Custom algorithm to compare the level of gene expression (p-value filter of < 0.05; either > 2 fold change or < 0.50 fold change). Ten genes were down-regulated and nine genes were up-regulated in CBA/SIV animals compared to SUC/SIV. The Search Tool for the Retrieval of Interacting Genes (STRING) and the Database for Annotation, Visualization and Integrated Discovery (DAVID) were used to determine the function of the genes. Gene expression changes suggest a compensatory response to inflammation due to CBA/SIV. Anti-inflammatory gene expression is increased, simultaneously decreasing expression of genes involved in cell growth. Supported by AA07577, AA09803, AA11290

Rats genetically expressing HIV-1 viral proteins exhibit attenuated cocaine-mediated increase in synaptosomal [3H]dopamine uptake in striatum following cocaine self-administration

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To understand how genetic expression of HIV-1 viral proteins influences the dopamine (DA) transporter (DAT) function, the current study explored the potential mechanism(s) underlying the alterations in DAT function of HIV-1 transgenic (Tg) rats in response to cocaine. In Experiment-1, the Vmax of [3H]DA uptake was increased (51%) in striatum of drug naive male Tg compared to Fisher 344 (F344) rats. Total DATs were not different between Tg and F344 rats but the cell surface DAT was increased (24%) in striatum of Tg rats. Tg rats showed decreased Bmax (24%) of [3H]WIN35,428 binding along with the increased Vmax. These results suggest that Tg rats exhibit neuroadaptive changes in striatal DAT function under basal conditions with an increased DA uptake turnover rate (Vmax/Bmax) and cell surface DAT expression, perhaps compensating for their damaged DAT function by HIV-1 viral proteins. In Experiment-2, ovariectomized female Tg and F344 rats underwent cocaine (0.33 mg/kg/infusion, FR-1) or sucrose (5% w/v) self-administration (SA) for 31 days. 24 hr after the final SA session, prior sucrose SA experience increased (41%) Vmax in striatum, but not in prefrontal cortex (PFC), of Tg rats relative to F344 rats. Compared to sucrose, F344 rats self-administering cocaine exhibited increased Vmax (60%) in striatum but not in PFC, whereas Vmax were not altered in striatum, but decreased by 70% in PFC, of cocaine self-administering Tg rats. Collectively, Tg rats represent a unique model for studying the effects of HIV-1 viral proteins on in vivo DAT function and cocaine-mediated behavior. Supported by Supported by NIH grants: DA035714, DA013137, HD043680, DA021287, and GM081740

Association of Mental Disorders with Cocaine Use in HIV-1 Infected Females in South Florida

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According to the Center for Disease and Control Prevention, in 2011, an estimated 49,000 new cases of HIV-1 infection were reported in the United States, of those 1,262 new cases were identified in Miami-Dade County alone (Florida Department of Health, 2011). It is well understood that drugs of abuse such as cocaine play a significant role in neuropathogenesis of HIV-1 infection and mental disorders. Although HIV-1 does not directly infect neurons, significant brain involvement occurs in individuals infected with the virus. We hypothesize that mental disorder is associated with cocaine use and gender differences. The most common mental disorders, such as Bipolar D/O and Schizophrenia Disorders and depression were studied among HIV infected cocaine using and non-using female and male patients. In this study we have performed a retrospective analysis of 139 HIV-1 infected male and female cocaine users. Our results show that mental disorder in HIV infected cocaine adults is significantly

associated in females than males. These results suggest that cocaine use is an important factor in the incidence of mental disorders in HIV infected patients, specifically in females. Supported by 1R01MH085259, 5R01DA021537, 1R337DA025576

Prolonged exposure to morphine induces cell adhesion in an in vitro model of the blood-brain barrier

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Opioid abuse by human immunodeficiency virus type 1 (HIV-1)-infected individuals leads to more rapid disease progression, increased viral replication and peripheral viral load, and increased incidence and severity of neurocognitive abnormalities compared to non-drug abusers. The blood-brain barrier (BBB) is an obstacle that must be overcome during neuroinvasion with subsequent development of HIV-associated neurocognitive disorders (HAND). Previous studies of mu-opioids and alteration of BBB permeability have suggested that exposure increases cellular transmigration through an uncharacterized mechanism. In this study, a human brain microvascular endothelial cell (hBMEC) line, hCMEC/D3, was used to establish an in vitro transwell model of the BBB to investigate the effects of chronic (24, 48, 72 h) morphine treatment on barrier structure and function. We observed that hCMEC/D3 cells formed a confluent monolayer with a basal rate of passage of a tracer molecule comparable to primary hBMECs. It has also been shown that these cells express mu opioid receptor, and that prolonged morphine treatment induces changes in mRNA levels of cellular adhesion molecules. Functionally, an increase in PBMC transmigration and firm adhesion was observed following prolonged morphine exposure, in the absence of an increase in overall barrier leakiness. These results have suggested that morphine activates hCMEC/D3 cells leading to a cell environment permissive to transmigration. These studies may uncover a mechanism by which morphine disrupts periphery-CNS homeostasis leading to accelerated HAND. Supported by This work is supported by NIH/NINDS R01 NS32092, NIDA R01 DA19807, NIMH P30 MH092177, and NIMH T32 MH079785.

Disruption of the blood-brain barrier is responsible for the neurotoxicity induced by methamphetamine exposure

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As a drug of abuse, methamphetamine (METH) induces working memory deficits, oxidative stress, neuroinflammation, hyperthermia, and disruption of the blood-brain barrier (BBB). While the hippocampus is one of the brain regions particularly susceptible to METH toxicity, the mechanisms underlying METH toxicity are still poorly understood. In particular, the input of METH-induced BBB disruptions on adult brain neurogenesis is not known. The central hypothesis of the present study is that the disruption of BBB integrity induced by chronic METH exposure is responsible for toxicity to neural stem cells (NSCs), resulting in deficient neurogenesis. To address this hypothesis, mice were treated with escalating doses of METH (first dose, 0.2 mg/kg; the last dose, 2.4 mg/kg) 3 times per day for four days. Exposure of C57BL/6 mice to METH reduced immunoreactivity of nestin, the marker protein of NSCs, in the dentate gyrus. Because the samples were collected 5 days after the last injection of METH, these results suggest that aberrant neurogenesis persists even exposure to METH is discontinued. Delayed differentiation was also observed in cultures of mouse neural stem cell line NE-4C and primary mouse embryonic NSCs treated with 10 μ M METH for 10 days. Indeed, METH exposure reduced the length of dendrites in both cell types and inhibited the formation of secondary branching dendrites in NE-4C cells. These results suggest that METH exposure affects differentiation of NSC into the proper neuronal lineage, which may contribute to the development of cognitive dysfunction in drug abusers. Supported by NIH grants: DA027569, MH072567 and MH098891 to MT; MH063022 and Miami Center for AIDS Research at the University of Miami to MP

Cocaine using HIV-1-infected African American individuals in the DREXELMED HIV/AIDS Genetic Analysis Cohort have altered immunomodulatory profiles

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This study evaluated the relationship between illicit drug use and HIV-1 disease severity in HIV-1-infected patients enrolled in the DREXELMED HIV/AIDS Genetic Analysis Cohort. Since cocaine is known to have immunomodulatory effects, the cytokine profiles of preferential non-users (PN), cocaine users (PCo) or multi-drug users (MDU) were analyzed to understand the effects of cocaine on cytokine modulation and HIV-1 disease severity. Patients within the cohort were assessed longitudinally for HIV-1 clinical parameters and history of illicit drug, alcohol, and tobacco use. The Luminex human cytokine 30-plex panel was used for cytokine quantification. Analysis was performed using a newly developed biostatistical model. Substance abuse was found to be common within the cohort. Utilizing the drug screens at the time of each visit, it was determined that the cohort could be categorized as PN, PCo, and MDU. The overall health of the PN population was better than that of the PCo population, with peak and current viral loads in PN substantially lower than those in PCo and MDU patients. Among the 30 cytokines investigated, differential cytokine profiles were established within the three populations. The Th2 cytokines, IL-4 and IL-10, known to play a critical role during HIV-1 infection, were positively associated with increasing cocaine use. Clinical parameters such as latest viral load, CD4+ and CD8+ T-cell counts, and CD4:CD8 ratio were also significantly associated with cocaine use. Based on these assessments, cocaine use appears to associate with more severe HIV-1 disease. Supported by This work is supported by NIH/NINDS R01 NS32092, NIDA R01 DA19807, NIMH P30 MH092177, and NIMH T32 MH079785.

SAMHD1 facilitates HIV-1 persistence: Role of miR-155

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HIV-1 productively infects microglia /macrophages, but astrocytes are generally restricted. The relative importance of the possible replication blocks in astrocytes, however, is yet to be delineated. Recently identified restriction factor, sterile alpha motif and histidine/aspartic acid domain-containing protein 1 (SAMHD1), has been shown to restrict HIV-1 infection in resting CD4 T cells and monocyte-derived dendritic cells. However SAMHD1 expression and HIV-1 restriction/persistence in CNS cells are yet to be studied. Previous studies have implicated the restrictive role of certain miRNA in HIV-1 infection in resting CD4+T cells; however their role and mechanisms in CNS cells have not been delineated yet. We hypothesize that varying SAMHD1 expression levels lead to restricted HIV infection and host miRNAs regulate SAMHD1 expression in CNS cells. We examined constitutive expression of SAMHD1 and miR-155a in astrocytes and microglia at the gene and protein level, their regulatory effects during HIV infection and the role of miR-155a on SAMHD1 regulation. Our results demonstrate that higher levels of SAMHD1 in astrocytes are associated with low levels of miR-155. Further, silencing of SAMHD1 with miR-155 mimics relieves the HIV-1 restriction by optimizing RT step through SAMHD1 regulation. In conclusion, our data suggest that effective combinations of both SAMHD1 and miR-155 along with HAART may completely eliminate CNS viral reservoirs thus opening novel avenues to improve current strategies to eliminate latent CNS reservoir. Supported by R01MH085259, R37DA025576

Aging impacts clinical and genetic markers of HIV-1 disease

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The introduction of HAART has resulted in significantly lower mortality rates, culminating in an HIV epidemic that increasingly affects older adults. Additionally, immune failure commonly occurs with both aging and HIV infection.

Current studies focus on the impact of age on LTR signatures that correlate with HIV-1 clinical parameters including CD4+ and CD8+ T-cell count and viral load (VL). A prospective, longitudinal study was conducted on 458 HIV-1-seropositive patients currently enrolled in the DREXELMED HIV/AIDS Genetic Analysis Cohort in Philadelphia, PA. History of illicit drug, alcohol, and medication use, CD4+ and CD8+ T-cell count, and viral load, along with patient history including age, were collected about every 6 months. Using a linear mixed model involving longitudinal data, it was determined that there were viral SNPs that significantly correlated with increasing age. Additionally, age modified the SNP effect with respect to CD4+ T-cell counts and VL to different degrees. Both HIV-1 infection and age are also known to have immunomodulatory effects. The cytokine profiles of adult and aged individuals were analyzed to understand the impact of age and HIV-1 infection on cytokine modulation and HIV-1 disease severity. Interestingly, among the 30 cytokines investigated, the cytokine profiles appeared different between these two populations. In addition, there appeared to be a number of SNPs that correlated with the age effect on cytokine levels. In conclusion, age, in the context of HIV-1 infection, appears to impact viral gene expression and immune activation. Supported by This work is supported by NIH/NINDS R01 NS32092, NIDA R01 DA19807, NIMH P30 MH092177, and NIMH T32 MH079785.

Morphine induced alterations in neuronal iron metabolism

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Ferritin Heavy Chain (FHC) is an iron sequestering protein and has a major role in modulating intracellular labile iron pool (LIP). FHC is also known as a negative modulator of CXCR4 activity. Recently, our lab has reported elevated FHC and disrupted CXCR4 signaling in the cortical neurons of postmortem brain tissues of HIV+ patients/drug abusers. Furthermore, our studies also reported that morphine induced FHC up-regulation inhibited homeostatic signaling of CXCR4 in rat cortical neurons both in vitro and in vivo. The present study is aimed at investigating morphine-induced alterations in LIP, ferric iron staining and expression of iron related proteins, transferrin receptor (TfR) and ferroportin (Fpn) in rat primary cortical neuron cultures at 24 hours after treatment. Alterations in LIP and ferric iron were assessed by calcein AM assay and enhanced DAB Perl stain respectively. Changes in the expression of TfR and Fpn were determined by immunofluorescent staining followed by multispectral imaging. Results revealed an increase in LIP that can be the cause for morphine induced neuronal FHC up-regulation. The increase in iron staining supports the fact that there is an increase in ferric iron (including that stored in FHC). Our preliminary observations showed a decrease in Fpn staining in the neurons with increased FHC, which partly explains the accumulation of intracellular LIP in the morphine treated neurons. Studies on morphine induced alterations in neuronal iron in vivo and the mechanisms by which morphine causes an increase in intracellular LIP are in progress. Supported by NIH grants DA32444 and DA15014 to OM

Delta-9-Tetrahydrocannabinol (THC) rescues mice from Staphylococcal Enterotoxin B (SEB)-induced Acute Lung Injury (ALI) and subsequent mortality by the induction of regulatory T-cells and the down-regulation of the miR-17-92 cluster.

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The superantigen Staphylococcal enterotoxin B (SEB) is a potent activator of V β 8+T-cells that results in the clonal expansion of ~30% of T-cells. Consequently, this leads to the release of inflammatory cytokines, induction of toxic shock, and eventually death. In the current study, we investigated if Delta-9-Tetrahydrocannabinol (THC), a marijuana cannabinoid known for its anti-inflammatory properties, could prevent SEB-induced mortality and alleviate Acute Lung Injury (ALI). Administration of SEB resulted in mortality, while THC treatment led to 100% survival. Additionally, THC treatment significantly decreased numerous SEB-mediated clinical parameters, such as production of inflammatory cytokines, immune cell infiltration into the lung, vascular leak, and improved airway resistance. Microarray analysis revealed that a prominent miRNA signature upon SEB exposure was the induction of the miR-17-92 cluster. Mechanistically, we found the miR-17-92 cluster targets Pten (phosphatase and tensin homolog), an inhibitor the PI3K/Akt signaling pathway, thereby promoting cellular proliferation and the suppression of T-regulatory cells. THC treatment, however, inhibited the miRNAs in the cluster. Moreover, in its ability to act as a PI3K/Akt inhibitor, THC treatment led to the induction of T-regulatory cells and suppressed cellular proliferation. We report, for the first time, a miRNA expression profile in response to SEB. Furthermore, our results suggest that THC is a potent anti-inflammatory compound that acts by modulating critical miRNA involved in SEB- induced toxicity and death. Supported by NIH grants P01AT003961, R01AT006888, R01ES019313, R01MH094755, P20RR032684 and VA Merit Award BX001357

Polydrug mediated effects on HIV Nef+ exosome release and neuropathogenesis is modulated by Nef peptide magnetic nanocarriers.

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Polydrug use is implicated in exacerbating HIV infection and accelerating NeuroAIDS progression. There is a high prevalence of cocaine use among opioid abusers. Injection of cocaine and heroin (speedball) alters HIV replication and pathogenesis. Identifying pathogenic mechanisms that underlie speedball abuse in HIV+ individuals is needed for effective therapeutic strategies. Negative effector (Nef), integral to HIV pathogenesis is released from nef-transfected or HIV-infected microglia in exosome-like microvesicles (exNef). exNef impairs integrity of the blood-brain-barrier (BBB) measured by transendothelial electrical resistance (TEER) and Dextran-FITC transport. Impact of speedball (Sb) on exNef, BBB integrity, and HIV replication is unknown. Here we show that Sb significantly modulated exNef release from nef-transfected microglia and synergized with exNef to up-regulate HIV transcription in astrocytes. Using an in vitro BBB model, Sb and exNef lowered and increased BBB permeability and TEER, respectively. We hypothesized that inhibition of exNef release could be a novel therapeutic target in HIV+ speedball abusers. Nef-transfected microglia (+/- Sb) were treated with a magnetic nanocarrier conjugated to Nef peptide (1-19) 24 hours post transfection. Supernatants were harvested, exNef isolated and Nef levels determined via anti-Nef ELISA. Significant reduction in exNef observed suggesting Nef peptides control exNef release. Overall, we showed a role of exNef in Sb exacerbation of HIV neuropathogenesis and demonstrated a nanomedicine-based therapeutic to control exNef. Supported by NIH; National Institute on Drug abuse 3R01DA027049-04S1

MAGNETIC NANOPARTICLE FOR TARGETTED ANTI-HIV DRUG DELIVERY IN *IN VIVO* MODEL

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HIV-1 remains one of the leading life-threatening diseases in the world. Present Highly Active Antiretroviral Therapy (HAART) has improved significantly the mean lifespan of HIV-infected patients. However, since many drugs have less penetrance into viral reservoir sites such as brain, development of an active targeted drug delivery system is essential to increase the efficacy of drug targeting. In this regard, we developed a multifunctional iron oxide magnetic nanoparticle (MNP) bound to anti-HIV drug azidothymidine 5'-triphosphate (AZTTP) (MNP-AZTTP) tagged with fluorescent probe for noninvasive imaging for targeting to mouse brain. MNP-AZTTP was encapsulated into liposomes with fluorescent probe incorporation. The drug dissolution study was done through dialysis at different time intervals (30min to 2 days) measured by HPLC. The initial in vivo characterization was carried out in mice by injecting MNPs through i.v. injection and retained within the 0.2 Tesla magnetic fields for 30min, 1hr, and 2hrs with anesthesia assistance. At the end, mice were sacrificed and brain tissues were harvested for co-localization studies of drug and MNP by fluorescent detection in brain tissue. Further, drug dissolution study displayed significantly sustained release property over free drugs. In vivo analysis indicated that within 2hr of injection the fluorescent MNP-AZTTP reached the brain while the signal from control group was negligible. This novel formulation will be able to deliver multiple anti-HIV drugs more efficiently to rodent brain through imaging process. Supported by 5R01DA021537 and 1R037DA025576

Treg depletion during active MCMV brain infection exacerbates post-encephalitic neuroinflammation

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Long-term, persistent inflammation of the central nervous system is commonly seen during neurodegenerative disease, stroke, and viral brain infection. Using a mouse model of murine cytomegalovirus (MCMV) infection, we have shown that the post-encephalitic brain is maintained in a state of chronic neuroinflammation, including the persistence of antibody-producing B-cells, chronic microglial cell activation, and retention of MCMV-specific memory CD8⁺ T-cells. This lingering neuroinflammation occurs concomitantly with accumulation and retention of immunosuppressive regulatory T-cells (Tregs) within the brain. The extent to which these Tregs help control prolonged immune activation following viral encephalitis is unknown. In this study, we used Foxp3-diphtheria toxin receptor (DTR)-GFP transgenic mice which, upon administration of low-dose diphtheria toxin (DTX), results in

specific depletion of naturally occurring Tregs. Sustained Treg depletion during active MCMV brain infection (0 - 7 days post infection), using scheduled injections of DTX, resulted in the exacerbation of post-encephalitic neuroinflammation. Notably, DTX-treated, MCMV-infected mice had increased microglial cell activation (major histocompatibility complex class II (MHCII) and programmed death ligand-1 (PD-L1) expression), CD4⁺ and CD8⁺ T-cell brain infiltration and retention, and proinflammatory mediator (iNOS and interferon- γ) expression. These data demonstrate that persistent post-encephalitic neuroinflammation is influenced by immunoregulatory Treg function during the acute phase of viral brain infection.

S100A8 secretion by $\Delta 9$ tetrahydrocannabinol-induced myeloid derived suppressor cells creates a positive feedback loop enabling continued accumulation and activation of MDSC

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With the recent legalization of marijuana for recreational purpose, in two states, as well as medicinal purpose, in 21 states, understanding the impact of cannabinoids is now paramount. We focused on the mechanism by which THC, the psychoactive ingredient found in the Cannabis sativa plant, induces MDSCs resulting in immunosuppression. However, the precise mechanism of THC-mediated MDSC induction and activation is not fully understood. In this study, we determined a mechanism through which THC generated and activated a highly immunoregulatory MDSC population. We found that THC-MDSCs had significantly increased levels of suppressive function, secretion of Stat 3 activating cytokines, and S100A8 protein compared to naive bone marrow resident MDSCs (BM-MDSCs). THC-induced granulocytic and monocytic MDSC subsets were more suppressive than BM-MDSC subsets. Additionally, arginase1 (Arg1), a known immunoregulatory molecule, was elevated in THC-MDSCs compared to BM-MDSCs. Increased levels of IL-6 and IL-10 in THC-MDSCs correlated with elevated Stat3 activation. S100A8 is a protein which has been shown in tumor models to drive MDSC accumulation and activation, but its role in THC-mediated MDSC induction is not known. Phosphorylated Stat3 has been shown to bind the promoter region of S100A8 and increase transcription. Use of the S100A8 depletion antibody (8H150) led to a 1.8 fold decrease in the accumulation of THC-MDSCs and impacted their suppressive function. Together, these data suggest a key role for S100A8 in THC-induced expansion and activation of highly suppressive MDSCs. Supported by NIH grants P01AT003961, R01AT006888, R01ES019313, R01MH094755, P20RR032684 and VA Merit Award

HIV-1/AIDS-Associated Neuropathic Pain: The Pathogenic Contribution of the gp120-Wnt-JNK-cytokine Cascade

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Neuropathic pain is a common neurological complication that severely deteriorates the life quality of HIV-1/AIDS patients, but effective therapies are not available. We are interested in understanding the pathogenic mechanism for the ultimate development of rationale-based therapeutic approaches. Our current focus is to identify the HIV-1 pathogenic factor and elucidate the molecular and cellular processes through which the factor causes neuropathic pain. We have undertaken an interdisciplinary approach in this research, including analyzing postmortem nervous tissues from HIV-1/AIDS patients, generating a clinically relevant rodent model, and determining the molecular, cellular, behavioral and electrophysiological abnormalities in the model. Our results indicate that gp120 is a causally relevant factor for the HIV neuropathic pain (Yuan et al. 2014 Annals of Neurology, under revision), that Wnt5a signaling is a critical downstream target that mediates the activity of gp120 in causing cytokine expression and glial reaction (Li et al. 2013, J Biol Chem.; Shi et al. 2013, JNIP; Shi et al. 2012, J Neurosci.), and that the expression of gp120-induced allodynia, neuropathy and SDH neuron sensitization depends on a Wnt5a-JNK-TNF- α pathway in the SDH (Yuan et al. 2014 manuscript in submission). The results collectively reveal a molecular pathway that is important for the pathogenesis of HIV-associated neuropathic pain. Supported by NINDS: R01 NS079166; NIDA: R01 DA036165

POST-INJURY ALCOHOL EXPOSURE AGGRAVATES NEUROPATHOLOGICAL SEQUELAE AND NEUROBEHAVIORAL OUTCOMES AFTER TRAUMATIC BRAIN INJURY

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Alcohol abuse is a significant risk factor in ~1.7 million reported traumatic brain injury (TBI) cases in the U.S. annually. Although ~26% of patients continue to use alcohol after TBI, few preclinical studies have examined the impact of alcohol exposure on TBI recovery. The aim of this study was to determine the effect of post-TBI alcohol exposure on neuropathological and neurobehavioral recovery. Adult male Sprague-Dawley rats were fitted with a female Luer-lock over a 5 mm left lateral craniotomy. TBI was induced by lateral fluid percussion (~30 PSI, ~25 ms). At day 4 post-TBI, rats were exposed to chronic intermittent alcohol vapor (CIA) or room air for 10 days (BAL~200 mg/dL). TBI produced apnea, delayed righting reflex, and increased neurological severity score (NSS). TBI/CIA group showed impaired NSS recovery throughout CIA exposure and abstinence. In addition, TBI/CIA impaired recognition memory, decreased locomotion, and increased anxiety in novel object recognition and open field exploration tests. Immunofluorescence revealed increased activation of astrocytes, microglia, toll-like receptors, and other markers of neuroinflammation in TBI/CIA brains. Furthermore, increased apoptosis was detected in alcohol exposed brains after TBI. These findings indicate that alcohol impairs neurobehavioral recovery after TBI, which may be caused by exacerbated neuroinflammation leading to increased neuronal death. The clinical implications for TBI patients exposed to alcohol during recovery warrant further investigation. Supported by NIAAAT32-AA007577, F30-AA022838

Immunopathogenesis of HIV-1 Infection in Cocaine users: Role of Arachidonic Acid

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Aim: Previous studies have demonstrated that HIV infections and drugs of abuse such as cocaine have been identified as risk factors for triggering HIV-1 disease progression and immune dysfunctions. Dendritic cells (DCs) are the first line of antigen presenting cells and defense against various diseases including HIV-1 infection. The synergistic effects of cocaine and HIV-1 infection and the potential role of arachidonic acid on immunopathogenesis of HIV-1 infection have not been clearly elucidated. We hypothesize that HIV infection and cocaine synergistically dysregulate the gene expression of arachidonic acid (AA) and its metabolites (PGE2, COX-2 and 5-LOX, 15-deoxy- Δ 12,14-PGJ2 (15d-PGJ2) and protein (14-3-3 ζ/δ) modifications leading to immunopathogenesis of HIV-1 infection. **Methods:** Immature dendritic cells (IDC) and plasma samples were used from HIV-1 positive and negative cocaine users and age matched normal control subjects. The AA metabolites COX-2, TBXA2R, 5-LOX gene and protein expression were studied in IDCs while PGE2, 15d-PGJ2 and 14-3-3 ζ/δ levels were measured in plasma samples by ELISA and western blot respectively. **Results:** Results indicated that plasma concentration levels of AA, PGE2 and COX-2, TBXA2R and 5-LOX in IDCs were significantly higher in cocaine using HIV-1 infected subjects whereas 15d-PGJ2 and 14-3-3 ζ/δ were significantly reduced compared to either HIV positive subjects or cocaine users. **Conclusions:** These results suggest that cocaine synergistically upregulate AA secretion and its metabolites exacerbating immunopathogenesis of HIV-1 infection. Supported by NIH- DA 025576 and MH08529.

Bluetongue Virus Activates TLR-3 and Inhibits HIV Infection of Macrophages

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Bluetongue virus (BTV) is a non-enveloped double-stranded RNA (dsRNA) virus that can activate Toll like receptor 3 (TLR3) signaling pathway, inducing interferon (IFN)-mediated antiviral activities. We thus investigated effect of BTV16 on HIV infection of human primary macrophages. We demonstrated that TLR3 activation of macrophages by BTV16 suppressed HIV infection. BTV16 treatment of macrophages induced both type I and III IFN expression, leading to the production of IFN inducible antiviral factors, including APOBEC3G/F/H (A3G, A3F, A3H), myxovirus resistance protein A (MxA), 2',5'-oligoadenylate synthetase (OAS-1), and the IFN-stimulated gene 56 (ISG56). In addition, BTV-treated macrophages expressed increased levels of CC chemokines (MIP1- α/β , RANTES), the ligands for HIV entry co-receptor CCR5. BTV16 also induced the expression of tetherin, intracellular factor that restrict HIV release from infected cells. Furthermore, TLR3 signaling of macrophages by BTV16 resulted in the induction of the microRNAs (miRNA-28, 29a, 125b, 150, 223, and 382) that inhibit HIV transcription. This BTV16-mediated enhancing effect on the expression of multiple HIV restriction factors were also observed in macrophages treated with UV-inactivated BTV16 (UV BTV16). The TLR3 inhibitor could completely block the effects of BTV16 or UV BTV16 on TLR3 signaling of macrophages. These findings demonstrate the importance of BTV-mediated TLR3 activation in macrophage innate immunity against HIV. Future in vivo investigations are necessary in order to determine the clinical application of BTV in treatment of HIV disease. Supported by NIH DA12815, DA27550, DA22173 and DA36163, NNSFC 81271334

Multimodal neuroimaging evidence of alterations in cortical structure and function in the aging HIV brain.

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Despite the availability of combination antiretroviral therapy, HIV-infected patients remain at an increased risk of developing neurocognitive disorders and the mechanisms are not understood. Some previous neuroimaging studies have found hyperactivation in association areas of HIV-infected patients, whereas others reported hypoactivation in sensory regions. In this study, we evaluate whether both patterns coexist in infected patients and whether these deficits reflect aberrations in brain structure, function, or both. HIV-infected adults and matched controls completed structural magnetic resonance imaging (sMRI) and a mechanoreception task during magnetoencephalography (MEG). MEG data was examined using beamforming and sMRI data was analyzed using the latest voxel-based morphometry methods. We found significantly reduced activity in the postcentral gyrus and increased activity in the prefrontal cortex of HIV-infected patients relative to controls. Patients also had reduced gray matter in the postcentral gyrus, parahippocampal gyrus, and other areas. Importantly, reduced gray matter volume in the postcentral gyrus was spatially-coincident with abnormal MEG activity. Finally, prefrontal and postcentral gyrus activity was correlated with neuropsychological function, and had a combined sensitivity and specificity of over 87% for HIV-associated cognitive impairment. This study is the first to demonstrate abnormally increased activity in association regions with simultaneously decreased activity in sensory areas. These MEG findings may hold promise as a potential disease marker. Supported by NIH grant P30 MH062261 (HSF) and a Kinman-Oldfield Award (TWW).

THC-mediated epigenetic modification leads to immune suppression by dysregulation in T helper cell differentiation

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Marijuana is the most common illicit drug in the United States. Meanwhile, there are increasing efforts to legalize marijuana. THC is the main bioactive component in marijuana and its activity is primarily mediated by cannabinoid receptors. Besides its psychopathological effect, THC has been shown to disrupt immune response. However, the mechanisms by which THC regulates gene expression in immune cells are not clear. Increasing evidence indicates that epigenetic modifications such as histone and DNA methylation play critical roles in regulating biological processes including immune response. Using ChIP-Seq technology, we sought to investigate whether THC alters global histone methylation pattern. We compared genome-wide histone H3K4, H3K27, H3K9 and H3K36 trimethylation patterns between THC and vehicle treated lymphocytes which were activated with staphylococcal enterotoxin. Our results show that THC treatment leads to the association of active histone methylation markers with Th2 cytokine genes and suppressive markers with Th1 cytokine genes. These results suggest that THC inhibits Th1 cells while promoting Th2 cells through histone modification. At the genomic level, a significant portion of histone methylation regions are altered by THC treatment. Functional classification of these histone methylation associated genes shows that these differentially associated genes are involved in various cellular functions, from cell cycle to metabolism, suggesting that THC regulates global gene expression in immune cells through histone modification. Supported by NIH, VA

Bath salts alter synaptic plasticity gene expression in neurons

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Bath salts which have been called as new LSD recently emerged as powerful and violent recreational drug in the market. Methylenedioxy pyrovalerone (MDPV) is the main cathinone found in Bath Salt. Previous studies have shown that MDPV use induces variety of psychoactive effects such as compulsive re-dose, anxiousness,

nervousness, paranoia, excessive excitation, and violent psychotic behavior including the recently reported cannibal attack that resulted in gnawing off another man's face in Miami. Synaptic plasticity is one of the molecular foundations of learning and memory. The effects of MDPV on synaptic plasticity gene expression, spine number and morphology etc. have not been reported yet. We have studied MDPV-mediated effects following acute and chronic treatments on synaptic plasticity genes in SK-N-MC, a neuronal cell line by RT Profile PCR Array. Several genes including JUN, JUNB, FOS, RAB3A, PPP1CA, etc. were significantly dysregulated following acute and chronic treatment of SK-N-MC with MDPV. Further, a significant reduction in the spine length, numbers, density and dendrite diameters were also observed after MDPV treatments. Our results provide first insights into the MDPV mechanisms of addiction and behavioral changes which may be associated with dysregulation of synaptic plasticity genes. Further, these studies may suggest molecular targets for the development of therapies against the MDPV induced drug addiction and neuropsychotic behaviors. Supported by 1R01DA027049 and 1R037DA025576

Beta-catenin rescues astrocytes from methamphetamine-induced cell senescence

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Methamphetamine (Meth), a potent psychostimulant that has exceeded cocaine abuse in the United States, causes oxidative stress in dopaminergic neurons but its potential effects in astrocytes are understudied. We evaluated the impact of Meth on the functional integrity of astrocytes. We demonstrate that Meth at a daily dose of 300uM induced senescence of primary human fetal astrocytes (HFA) by day six, as demonstrated by 3-fold increase of cells positive for the senescence-associated beta-galactosidase (SA-beta-gal) and 2-fold increase in p16INK4a, a critical regulator in the induction of cellular senescence. Meth-mediated induction of HFA senescence was dose dependent. Because we previously demonstrated that Meth down regulates beta-catenin level in astrocytes and given that beta-catenin is a pro-survival and pro-proliferative signal, we evaluated whether beta-catenin over expression in Meth-treated HFA can rescue these cells from cellular senescence. Transfection of HFAs with a constitutively active form of beta-catenin (S35Y beta-catenin) blocked Meth-induced astrocytic senescence by approximately 10-fold. These studies indicate that Meth induces astrocyte senescence and that beta-catenin can rescue astrocytes from Meth induction of cell senescence. This work is supported by 1 R01 DA033966-01A to LA. Supported by National Institute on Drug Abuse (NIDA)

HIV Infection and Heroin Use Dysregulate the Circulating miRNA Expression

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Injection drug users (IDUs) represent one of the largest reservoirs of HIV infection in the United States. Cell-free circulating microRNAs (miRNAs) secreted by peripheral blood cells or specific organs hold great promise as a new group of biomarkers because of their high stability and ease of measurement. Recent studies have suggested that some of these miRNAs are related to HIV infection and AIDS progression. Yet little is known about the impact of drugs of abuse on the expression of circulating miRNAs in HIV-infected or uninfected IDUs. Thus, we investigated the impact of HIV infection and/or heroin use on the expression of the circulating miRNAs in vivo. We examined a total of 8 miRNAs that have been shown to be related to host innate immunity and HIV infection. Among the circulating miRNAs examined, miRNAs-29a, 198 and 382, which were reported to possess inhibitory effect on HIV replication, were significantly down-regulated in HIV-infected subjects. The levels of miRs-198 and 382 were also decreased in the heroin users. In addition, HIV infection suppressed the expression of several cellular anti-HIV miRNAs, miRs-28, 125b, 150 and 382 in macrophages. These findings provide evidence that HIV infection and/or heroin use negatively regulate the expression of anti-HIV miRNAs at both secreting and cellular levels, which may account for the dysfunction of innate antiviral immunity and HIV infection/ persistence among IDUs. Supported by DA012815 and DA022177 to Dr. Wenzhe Ho