Brain amyloid and HAND in the cART era

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HIV-infected individuals may be at increased risk of accelerated aging and thereby age-dependent conditions such as sporadic Alzheimer's disease (AD) and cerebral small vessel disease (CSVD). Using a clinico-pathological approach, we demonstrated that apolipoprotein (APOE)ε4 genotype and older age (≥50 years) independently increased the occurrence of isocortical diffuse β-amyloid (Aβ) plaques, while phospho-Tau neurofibrillary lesions were sparse in the isocortex. Therefore, these changes in HIV brains were different from those found in symptomatic AD brains. Furthermore, the presence of Aβ plaques were associated with HAND among APOE ε4 carriers. The detection of APOE ε4 genotype and cerebral Aβ deposition biomarkers in CSF may be useful in identifying living HAND patients who could benefit from Aβ-targeted therapies. Regarding CSVD, we found that protease-inhibitor-based antiretroviral therapy increased the risk of arteriolosclerosis after adjusting for diabetes, and HAND was associated with mild arteriolosclerosis. In conclusion, whether HIV-infected individuals are at greater risk of cerebral β-amyloidosis or AD, relative to non-HIV subjects, remains to be determined, although we have found in several brains from older HIV+ individuals neuropathologic changes diagnostic of AD. Furthermore, we believe that studies to confirm the benefit of CSF biomarkers for cerebral β-amyloidosis and CSVD in predicting HAND are warranted.

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Enhancement of HIV-1 Integration in CD4+ T cells by Cocaine

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Epidemiological studies suggest that cocaine abuse worsens HIV-1 disease progression. Although the mechanism remains unclear increased viral load has been

suggested to play a key role for the accelerated HIV disease among cocaine abusing patients. Therefore, our goal was to investigate whether cocaine enhances HIV-1 integration as a potential mechanism to increase viral load. We infected CD4+ T cells that are the primary targets of HIV-1 in vivo and treated the cells with physiologically relevant concentrations of cocaine (1-100μM). Viral DNA integration in these cells was measured by quantitative PCR. Our results illustrated that cocaine from 1-50μM increased HIV-1 integration in CD4+ T cells in a dose dependent manner. Increased integration can also be achieved by modulating several early steps of HIV-1 replication. Cocaine increased integration of pseudotyped virions illustrating that cocaine's effects may not depend on increased viral entry. To further examine direct effects of cocaine on viral integration we conducted in vitro integration assays using HIV-1 preintegration complexes. Our data confirmed that cocaine directly increased viral DNA integration. Our mass spectrometry analysis showed that cocaine is able to enter CD4+ T cells and localize to the nucleus- the site of viral integration. In summary, our data provide strong evidence that cocaine can increase integration in CD4+ T cells. Therefore, we hypothesize that increased HIV-1 integration is a novel mechanism by which cocaine enhances viral load and worsens disease progression in drug abusing HIV-1 patients.

Supported by Enhancement of HIV-1 Integration in CD4+ T cells by Cocaine

Ex-vivo and in-vitro evidence of epigenetic mechanisms of alcohol abuse

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Histone modifications are crucial for gene regulation; for instance, histone deacetylases (HDACs) have been shown to play a role in addiction through regulation of genes involved in substance abuse. Previous reports have demonstrated that alcohol induces HDAC2 in CNS cells; however, the effects of alcohol on other HDACs and their immunomodulatory effects have not been elucidated yet. Therefore, the aim of the current study is to investigate the epigenetic mechanisms involved in monocyte-derived dendritic cell (MDDC) modulation in the context of alcohol abuse by performing qRT-PCR, western blot, and flow cytometry to analyze gene and protein expression of class I

HDACs by MDDC from two study groups: healthy controls and alcohol abusers. HDAC activity was also measured in MDDC from alcohol abusers. The ex-vivo results were validated with in vitro alcohol treatments (0.1% and 0.2%) of MDDC. In addition, H3 and H4 histone modifications were also analyzed by multiplex colorimetric assay. Our results show HDACs expression and enzymatic activity are differentially regulated across the groups with higher levels of expression shown in the alcohol abusing group and alcohol treated cells. While the effects of HDACs in the context of alcohol abuse are poorly understood, our results suggest that alcohol induces epigenetic mechanisms that may trigger addiction-related genetic modifications. Our findings contribute to a better understanding of the genetic HDAC profiles of alcohol abusing patients, which may lead to the development of new approaches to treat alcohol abuse disorders.

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Effect of methamphetamine on spectral binding and ligand docking of anti-HIV drugs with CYP3A4

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Cytochrome P450 3A4 (CYP3A4) is the major drug metabolic enzyme, and is involved in the metabolism of antiretroviral drugs, especially protease inhibitors (PIs). This study was undertaken to examine the effect of methamphetamine on the binding of PIs with CYP3A4. We showed that methamphetamine exhibits a type I spectral change upon binding to CYP3A4 with δAmax and KD of 0.016±0.001 and 204±18 µM, respectively. Further, we studied the effect of methamphetamine binding on PIs with CYP3A4. Our results showed that methamphetamine alters spectral binding of type I PI, nelfinavir, to CYP3A4 by decreasing both the δAmax (0.004±0.0003 vs. 0.0068±0.0001) and KD (1.42±0.36vs.2.93±0.08 µM). However, methamphetamine did not alter the binding of other type I PIs with CYP3A4 (lopinavir, atazanavir, tipranavir). Furthermore, the results showed that methamphetamine alters the binding of type II PIs to CYP3A4 by decreasing both the δAmax (0.0038±0.0003 vs. 0.0055±0.0003) and KD (0.043±0.0001 vs. 0.065±0.001 nM) of ritonavir, while decreasing only the KD of indinavir (0.086±0.01 vs. 0.174±0.03 nM). Finally, CYP3A4 docking with lopinavir and ritonavir in the absence

and presence of methamphetamine showed that methamphetamine alters the docking of ritonavir. Overall, our results demonstrated differential effects of methamphetamine on the binding of PIs with CYP3A4. These findings have clinical implication in terms of drug dose adjustment of antiretroviral medication in HIV-1-infected individuals who abuse methamphetamine.

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Gene expression changes associated with HIV-induced nuclear translocation of amyloid beta in brain endothelium

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Increased amyloid deposition in HIV-1-infected brains may have a role in the pathogenesis of neurocognitive dysfunction in infected patients. We have previously shown that exposure to HIV results in enhanced amyloid β (Aβ) levels in human brain microvascular endothelial cells, suggesting that brain endothelial cells contribute to accumulation of Aβ in HIV-infected brains. Importantly, Aβ not only accumulates in the cytoplasm of HIV-exposed cells but also enters the nuclei of brain endothelial cells. Moreover, inhibition of dynamin by dynasore effectively attenuated the HIV-1-induced Aβ nuclear uptake. To explore the possible mechanisms involved, we performed cDNA microarray analysis in order to examine changes in the transcriptional profile associated with this phenomenon. Gene network analysis indicated that inhibition of nuclear entry of Aβ resulted in enrichment in gene sets involved in apoptosis and survival, endoplasmic reticulum stress response, immune response, cell cycle, DNA damage, oxidative stress, cytoskeleton remodeling and transforming growth factor b (TGFβ) receptor signaling. Data from this study suggest that blocking HIV-1-induced Aβ nuclear uptake may involve, at least partly, several cellular stress related pathways that may be relevant for HIV-1 induced Aβ pathology.

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Brain endothelial derived extracellular microvesicles (eMVs) as biomarkers of BBB remodeling during neuroinflammation: Implications for cerebrovascular injury in substance abuse

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Inflammatory mediators, pro-oxidative stress agents, biomechanical forces and exposure to commonly abused drugs can alter the function of the blood-brain barrier (BBB). These induced changes could result in BBB hyper-permeability or 'leakiness'. We hypothesized that detection of extracellular microvesicles (eMVs), shed by brain endothelial cells offers the potential to monitor BBB health and remodeling. Here we present results related to the composition and degree of eMV release from primary human brain endothelial cells exposed to a broad range of insults. Our panel simulates conditions that are known to affect BBB function, such as pro-inflammatory cytokines and changes in fluidic shear stress and strain. In addition, since it is known that BBB dynamics are sensitive to psychostimulant drugs; eMV biogenesis in BMVECs exposed to cocaine and methamphetamine was also analyzed. Our findings show significant differences, as a function of insult, in both the levels of eMVs and their compositional matrix of endothelial and exosome markers, and tight junction proteins (TJP). Furthermore, cocaine-mediated BBB remodeling was investigated in plasma samples from mice showing cocaine induced conditioned place preference. Mice in the cocaine groups had elevated levels of eMVs containing TJPs, claudin-5 and occludin, and ARF-6 (a GTP-binding protein linked to eMV formation). These studies aim to bring further insight into the process of brain vascular remodeling and offer the potential for biomarker assay development for evaluation of BBB status in a broad range of CNS pathologies.

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POTENTIAL ROLE OF PURINERGIC P2X7 RECEPTORS IN THE PROGRESSION OF ALCOHOL INDUCED BRAIN AND LIVER DAMAGE

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Neuroinflammation and neurodegeneration contribute to chronic alcohol related brain damage. ATP-gated purinergic P2X7 receptors (P2X7Rs) are implicated in inflammation and in the development of neurodegenerative pathologies. Our recent findings supported a role for P2X7Rs in ethanol-induced neuroinflammation and neurotoxicity. We found increased P2X7R protein levels with parallel neuroinflammatory response and neurotoxicity in ethanol-sensitive brain regions, striatum and hippocampus, using a model that combines intragastric ethanol feeding and high fat diet (Hybrid) in C57BL/6J mice for 8 weeks. The Hybrid paradigm has been developed to study alcoholic liver disease. The present study tests the hypothesis that P2X7R plays a role in the progression of ethanol-induced neuroinflammation and liver pathology. Mice were exposed to Hybrid paradigm for 2 and 4 weeks, brain and liver tissues were harvested to test for P2X7R protein expression, neuroinflammatory response and histological changes. Hybrid exposure for 2 weeks caused an increase in P2X7R protein expression in striatum which was aggravated after ethanol withdrawal. Similar changes were found in livers of 2 week treated mice. Tests are underway to determine if changes in P2X7R levels parallel inflammatory responses. Overall, the initial findings suggest that P2X7Rs play a role in the progression of ethanol-induced brain and liver damage. Studies using P2X7R antagonists should help validate P2X7Rs as new potential therapeutic targets for the prevention and/or treatment of alcohol-induced brain and liver pathology.

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Methamphetamine treatment and HIV-1 infection dysregulate Synaptic plasticity in SKNMC neuronal cells

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Spread of HIV infection and drug abuse are significantly interlinked. Drug abuse can alter the neuroplasticity and damage the CNS analogous to that happens during the HIV infections. Many illicit drugs have been shown to promote susceptibility/progression of HIV infections and associated neuropathogenesis. US is currently experiencing a grave epidemic of methamphetamine (Meth) use entangled with HIV infection. Nevertheless, effects of Meth on individual synaptic plasticity genes and spine density in different neuronal cells have little been studied. As such, we sought to explore the effect of Meth on synaptic plasticity genes and spine density in neuronal cells. We analyzed the effects of HIV-1 infection and Meth treatment on the expression of 84 key human synaptic plasticity genes by SK-N-MC neuronal cells. The fold change in the gene expression profile was analyzed and compared to untreated cells. Out of 84 human synaptic plasticity genes interrogated, 8 key synaptic plasticity genes were significantly (>3 fold) down-regulated during treatment of Meth alone. HIV infection resulted in down-regulation of 23 key synaptic plasticity genes where co-treatment of Meth resulted in additive down-regulation of at least 19 genes. In confocal microscopy, we have observed altered spine density in HIV infected neuronal cells in combination with Meth. These data suggest that certain specific synaptic genes are being down regulated by HIV or Meth which could play an important role in HIV- and Meth induced neurodegeneration.

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Dysregulated IL12 expression in the peripheral circulation of ischemic stroke patients is regulated by multiple epigenetic mechanisms.

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Ischemic stroke (IS) is one of the leading diseases to cause death and disability but very poorly understood in the context of epigenetic mechanisms in regulating the genes involved. Epigenetic mechanisms like histone modification, DNA methylation and role of miRNAs are now understood to play a very crucial role in the regulation of gene expression. Various genes of different inflammatory pathways are also shown to be regulated by epigenetic mechanisms. Many of the inflammatory cytokines are reported to be dysregulated in clinical stroke settings. In the present work, we report the dysregulated expression of IL12 in the PBMCs from IS patients and provide evidence for a possible role of epigenetic mechanisms that might control the dysregulated expression. We looked into the methylation status of four histone markers (H3K4me3, H3K36me3, H3K9me3 and H3K27me3) and DNA methylation. Moreover, we provide evidence that miRNAs also play a role in the regulation of IL12 expression in IS pathology. We show that many miRNAs (hsa-miRNA-874-3p, -3609, -4675 etc.,) are down regulated in IS and they target IL12A and IL12B. Overall, our data gives a clue that IL12 dysregulation in the peripheral cells of IS patients is, partly if not solely, controlled by different epigenetic mechanisms. (This work was supported in part by National Institutes of Health grants P01AT003961, R01AT006888, R01ES019313, R01MH094755, and P20GM103641 as well as by Veterans Affairs Merit Award BX001357).

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Morphine induced bile acid imbalance leads to gut barrier compromise and systemic inflammation.

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Despite being predominant drugs of choice for anti-nociception, morphine and its pharmacological derivatives result in severe co-morbidities studied in numerous disease models in mice and humans due to peripheral side effects. Opioids have been shown to

promote gram-positive bacterial translocation across the gut mucosa, leading to systemic inflammation and sepsis in a TLR2 dependent manner. We have also shown that bacterial translocation due to the gut mucosal barrier compromise are a part of the commensal flora. In this study, we show for the first time that morphine fosters significant gut microbial dysbiosis and altered cholesterol/bile acid metabolism in WT mice. Recent studies have strongly correlated microbial/bile-acid dysbiosis to gut barrier disruption and host inflammation. In this context, role of hepatic cholesterol-7α-hydroxylase (CYP7A1) and Farnesoid-x-receptor (FxR; hepatic and intestinal) have been strongly implicated in complications arising due to bile acid imbalance. Here, we show the role of bile acid changes due to chronic morphine in gut barrier dysfunction in the context of TLR2/cholesterol hydroxylase/farnesoid-x-receptor modulation.

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Dysregulation of ER stress and autophagic responses by the anti-retroviral drug Efavirenz.

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Increasing evidence demonstrates that the anti-retroviral drugs (ARVds) used for HIV treatment have toxic effects resulting in various cellular and tissue pathologies; however, their impact on the cells composing the blood-brain barrier (BBB) is poorly understood. The current study was focused on ARVds, used either in combination or alone, on the inductions of ER stress responses in human brain endothelial cells. Among studied drugs (Efavirenz, Tenofovir, Emtricitabine, Lamivudine, and Indinavir), only Efavirenz increased ER. At the same time, Efavirenz diminished autophagic activity, a surprising result as induction of ER stress is typically linked to enhanced autophagy. These results were confirmed in microvessels of HIV transgenic mice chronically administered with Efavirenz. In a series of further experiments, we identified that Efavirenz dysregulated ER stress and autophagy by blocking the activity of the Beclin-1/Atg14/PI3KIII complex in regards to synthesis of phosphatidylinositol 3-phosphate (PI3P), a process which is linked to the formation of autophagosomes. Furthermore, we demonstrated that this disruption results in an increased endothelial permeability and a reduction in expression of tight junction proteins. Because of the

protective nature of the BBB, the disruption of the autophagy by Efavirenz and its impact of endothelial integrity may impact the development of neurodegenerative disease in HIV patients treated with this drug.

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A potential gut microbiome therapeutic approach for HIV-1+ drug abusers

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Equol is an active metabolite of the soy isoflavone daidzein, produced by the gut microflora during digestion. Equol is more potent than daidzein, prevents synaptodendritic damage produced by exposure to HIV-1 Tat and cocaine, and improves performance on executive function tasks in the HIV-1 Tg rat. Female ovariectomized F344 (F344E n=11; F344V n=10) and HIV-1 Tg (TgE n=11; TgV n=10) rats were used to evaluate equol (0.2mg) on HIV-1 mediated changes in motivation. First, we evaluated the effects of daily oral equol treatment on the incentive value of sucrose using fixed ratio (FR) and progressive ratio (PR) schedules of reinforcement (1,3,5,10, and 30% sucrose solutions; w/v). Equol treatment resulted in a significantly altered response for increasing concentrations of sucrose regardless of genotype, but HIV-1 Tg rats responded significantly less overall. Then, the effects of oral equol treatment every other day on 5 day FR (0.2mg/kg/inj) and 14 day PR (0.75mg/kg/inj) tasks were evaluated. Equol treatment differentially affected cocaine responding in HIV-1 Tg rats vs F344 rats (TgEF344V). Finally, we examined the potential long lasting effects of equol using a PR schedule (0.01-1.0mg/kg/inj) for 12 days. Chronic exposure to HIV-1 viral proteins altered response vigor for cocaine and sucrose. Active equol treatment had beneficial effects on sensitivity to cocaine and alters responding for sucrose, suggesting the manipulation of gut metabolites may be a novel therapeutic route for treatment of HIV-1 associated neurocognitive disorders and drug abuse.

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Depressive Symptoms and Inflammation During Breast Cancer Treatment

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Women undergoing treatment for breast cancer endure multiple stressors and may manifest depressed mood during treatment. As prior research has related depressed mood and depressive symptoms to indicators of inflammation in healthy populations, and since inflammatory processes may promote breast cancer disease progression, our lab has investigated the association between inflammatory indicators and depressive symptoms and depressive mood in women undergoing primary treatment for non-metastatic breast cancer. We recruited women in the weeks after surgery and prior to adjuvant treatment, conducted clinical interviews and collected blood samples and questionnaires. We found that women with clinically elevated depressive symptoms showed significantly greater levels of pro-inflammatory cytokines levels in serum, even after controlling for age, time since surgery and body mass index. We also found that negative affect (includes depressed mood) was associated with greater leukocyte pro-inflammatory and pro-metastatic gene expression, after controlling for sociodemographic, and clinicopathological covariates. The next steps are to conduct cellular and molecular studies of different immune cell subsets to examine the loci of these depression-inflammation associations in women at this early point in breast cancer treatment and relate these to longer-term health outcomes. We are also investigating whether cognitive behavioral interventions designed to improve stress management skills can modulate mood and inflammatory signaling in the short term and health outcomes in the long-term.

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MORPHINE INDUCES SYNAPTODENDRITIC IMPAIRMENT IN CULTURED HIPPOCAMPAL NEURONS: REVERSAL WITH PLATELET-DERIVED GROWTH FACTOR

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Chronic exposure to morphine often leads to reductions in spine density culminating into functional impairment of learning and memory. Strategies aimed at reversing the synaptodendritic injury are thus warranted in the field. PDGF is a pleiotropic growth factor involved not only in embryonic development but plays a vital role also in neuronal proliferation/differentiation during adulthood. Previous studies have shown the neuroprotective role of PDGF via the induction of synaptic plasticity gene Arc. The goal of this study is to explore the role of morphine in mediating synaptodendritic injury and the neuroprotective role of PDGF-BB in restoring the defect. In primary hippocampal neurons morphine reduced the spine density and excitatory synaptic marker vGlu with a concomitant upregulation of inhibitory synaptic marker GAD6. Morphine also elicited production of reactive oxygen species, molecular initiators for ER stress and autophagic markers. Pharmacological blockade approach suggested involvement of mu-receptor, oxidative stress, ER stress and autophagy in morphine-mediated synaptic injury. Furthermore, PDGF restored morphine-associated synaptic injury by increasing spine density and rescuing the loss of vGlu while suppressing the upregulation of GAD6 mediated by morphine. These findings underscore the important roles of oxidative stress, ER stress and autophagy in morphine-mediated synaptic impairment. PDGF could thus be considered as a therapeutic strategy for ameliorating morphine-associated cognitive impairment.

Dopamine Increases CD14+CD16+ Monocyte Transmigration Across the Blood Brain Barrier: Implications for Substance Abuse and HIV Neuropathogenesis

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Substance abuse accelerates the development and increases the severity of HIV associated neurocognitive disorders (HAND), but HIV neuropathogenesis in the drug abusing infected population is not well characterized. Peripheral blood CD14+CD16+ monocytes are increased with HIV infection. Transmigration of this mature monocyte subpopulation across the blood brain barrier (BBB) contributes to HIV entry into the CNS and to the establishment and propagation of chronic neuroinflammation, which mediate HAND. We demonstrate that the percentage of CD14+CD16+ monocytes in the periphery increases with drug use in HIV infected individuals. Dopamine, increased in the brain in response to drug use, enhances the transmigration of uninfected and HIV infected CD14+CD16+ monocytes, but not T cells, across our in vitro model of the human BBB. This transmigration is mediated by D1-like dopamine receptors as indicated by greater expression of D1R and D5R on CD14+CD16+ monocytes as compared to T cells and the ability of the D1-like dopamine receptor agonist, SKF 38393, to increase CD14+CD16+ monocyte transmigration across the BBB. Pseudopod formation and active ADAM17 expression by CD14+CD16+ monocytes, but not T cells, are increased by dopamine and may contribute to the ability of CD14+CD16+ monocytes to transmigrate. Thus, uninfected and HIV infected CD14+CD16+ monocyte entry into the CNS may increase with substance abuse by a novel mechanism mediated by D1-like dopamine receptor activation, contributing to neuroinflammation and the development of HAND.

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Cathepsin B and serum amyloid p component contribute to HIV-induced neuronal apoptosis

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Cathepsin B, a lysosomal protease, is actively secreted by HIV-infected macrophages and has been associated to neuronal apoptosis in vitro. We hypothesized that cathepsin B interacts with other macrophage-secreted proteins to trigger neuronal apoptosis. To elucidate the mechanism of neurotoxicity, we immunoprecipitated cathepsin B from uninfected and HIV-infected macrophage supernatants and identified interacting proteins by tandem mass spectrometry. Proteins with differences in spectral count were validated by western blot. The contribution to neuronal apoptosis was assessed by addition of uninfected and HIV-infected macrophage supernatants, pre-treated with antibodies against cathepsin B and interacting proteins, to SK-N-SH neuroblastoma cell line cultures, and using TUNEL labeling. Interestingly, serum amyloid p component (SAPC) co-immunoprecipitated with cathepsin B from HIV-infected supernatants. Antibodies against cathepsin B and SAPC decreased HIV-induced neuronal apoptosis by 10%. We observed by immunohistochemistry that cathepsin B and SAPC expression are increased in the brain of patients with HIV-subsyndromic disorder and HIV-associated dementia compared to controls. Both proteins co-localized with amyloid beta peptides in tissues from HIV-positive neuro-impaired patients and Alzheimer's disease patients. Cathepsin B and SAPC possible interaction in HIV-infected macrophage supernatants contribute to neuronal apoptosis and their overexpression in the brain of neurocognitive impaired patients suggest a role of this protein complex in neurodegenerative diseases.

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EFFECT OF METHAMPHETAMINE AND HIV-1 GP120 ON AUTOPHAGY IN SVGA ASTROCYTES

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Methamphetamine (METH), a commonly used controlled substance, is known to exacerbate neuropathological dysfunction in HIV-infected individuals. The neuropathological manifestation results from cell death in the CNS wherein autophagy is expected to play an important role. Autophagy is an intracellular activity that eliminates cytoplasmic organelles during deprivation/stress to protect cells from apoptotic cell death. In addition, excessive autophagy can be destructive, leading to autophagic cell death. This study was designed to investigate whether METH and HIV-1 gp120 interact to induce autophagy in SVGA astrocytes and cause increased cell death. We found that METH and gp120IIIb caused an increase in LC3II level in astrocytes in a dose- and time-dependent manner, and the level of LC3II was further increased when the cells were treated with METH and gp120IIIb simultaneously. This result was confirmed by detecting LC3II level in astrocytes using confocal microscopy and transmission electron microscopy. Next, we sought to explore the mechanism by which METH and gp120 induce the autophagic response. We found that signaling proteins PI3K, mTOR, Bcl-2, and Beclin-1 were involved in METH and gp120 mediated autophagy. In addition, chronic treatment with METH and gp120IIIb resulted in cell death in a synergistic manner. We are now in the process of determining the role of autophagy in METH and gp120-induced cell death. This study is novel and clinically relevant because METH abuse among HIV-infected populations is highly prevalent and is known to cause exacerbated neuroAIDS.

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Occludin regulates glucose uptake and epigenetically regulates inflammatory response in BBB pericytes

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Damage to the blood-brain barrier (BBB) is a common occurrence in a number of pathologies, which result in local and systemic inflammation. While the role of endothelial cells and astrocytes in BBB functions is well documented, the cell biology of pericytes and their role in the BBB remains poorly understood. Here we show that occludin, a protein typically associated with tight junctions in BBB-endothelial cells, but also expressed in pericytes and astrocytes, influences glucose uptake and expression of glucose transporters GLUT1 and GLUT4. These actions were most prominent in pericytes. Mechanistically, occludin-regulated glucose metabolism was linked to expression and activation of AMP-activated protein kinase (AMPK). In addition, occludin levels correlated with histone rearrangement, nuclear translocation of CtBP1 (gene repressor), and modified expression/activation of stress-response transcription factors (NFkB, SP1 and FOXO3). Transient exposure to TNFa (proinflammatory condition), resulted in occludin downregulation in pericytes and astrocytes, which was associated with decreased glucose uptake and GLUT1/GLUT4 expression. Paradoxically, threshold occludin levels were needed for these events to occur, as occludin silencing resulted in elevated glucose uptake upon TNFa treatment. Altogether our results show that occludin plays an important role in modulating metabolic responses in pericytes by epigenetically regulating gene expression and glucose metabolism. These findings provide a novel perspective in understanding pericyte metabolism and the BBB response to stress.

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Cocaine Self-Administration by HIV-1 Transgenic Rats Potentiates the Pathophysiology in the Nucleus Accumbens Core Induced by Cocaine or the Genotype Alone

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HIV-1 transgenic (Tg) rats express 7 of 9 HIV-associated toxic proteins. To develop a novel model of HIV/AIDS, addiction co-morbidity, we trained Tg rats to self-administer cocaine (COC-SA). To ascertain if the co-morbid condition exacerbates cocaine-induced pathophysiology which endures in brain reward circuits, including the nucleus accumbens core (NAc), we assessed excitability of the NAc core in adult male Tg and non-Tg rats with COC-SA or saline-yoked controls (SY). To do so, primary NAc neurons (medium spiny neurons; MSN) were recorded in ex vivo forebrain slices harvested 14 days after the last operant session using whole-cell patch-clamp technique. We found that MSN from COC-SA non-Tg rats showed significantly-reduced firing as compared to those from non-Tg SY rats. In contrast, MSN from SY Tg rats exhibited increased firing in response to moderate depolarizing currents, as compared to SY non-Tg rats, and this effect was potentiated by COC- SA. Changes in membrane properties (e.g., resting membrane potential, input resistance, and rheobase) support the findings that MSN excitability was reduced in COC-SA non-Tg rats, but was increased in Tg rats with or without COC-SA. This represents the first evaluation of NAc neuropathophysiology in Tg rats and the findings reveal that the NAc is functionally dysregulated after chronic exposure to COC and HIV-1 proteins in vivo. Study outcomes suggest that HIV proteins render NAc reward circuits more vulnerable to excitatory stimuli, especially when co-morbid with chronic cocaine exposure.

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ENDOLYSOSOME INVOLVEMENT OF HIV-1 TAT INDUCED NEURONAL AMYLOID BETA PRODUCTION.

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Increased life span of HIV-1 infected population is accompanied by increased prevalence of HIV-1 associated neurocognitive disorder (HAND), among which a significant incidence of Alzheimer's disease (AD)-like pathology, such as increased deposition of amyloid beta (Aβ), has been revealed. Although the underling mechanisms are not fully understood, HIV-1 transactivator (Tat) protein has been implicated in increased neuronal Aβ generation. Our recent findings that Tat disrupts

endolysosomal function, an early pathological feature of AD, prompted us to test the hypothesis that endolysosomal dysfunction is associated with Tat induced increases in neuronal Aβ generation. In primary cultured rat hippocampal neurons, endolysosome morphology was determined with LysoTracker and endolysosome pH was measured ratiometrically with a LysoSensor. Aβ was quantified with ELISA and protein distribution of APP, Aβ, and BACE-1 was measured with immunostaining. BACE-1 activity was measured with an enzyme activity assay. We demonstrated that Tat enlarged endolysosomes and elevated endolysosomal pH. We found that such disturbed endolysosomal dysfunction preceded Tat-induced increases in Aβ generation. In addition, we demonstrated that Tat increased endolysosomal accumulation of APP and Aβ as identified with 4G8 antibodies. Furthermore, we demonstrated that Tat treatment increased endolysosomal accumulation of BACE-1 and enhanced BACE-1 activities. Our findings suggest that Tat increases neuronal Aβ generation and contributes to the development of AD-like pathology in HIV-1 infected individuals

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Marijuana up-regulates HIV replication in microglia-like cells and restricts exNef- modulation of glia immune function.

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Marijuana has been shown to slow HIV-associated immunopathology in the periphery. However, the effect of marijuana exposure on HIV replication in the brain and immunopathology is unknown. Here we investigated the impact of marijuana on HIV replication and immune function of microglia, the immune cell of the brain. Microglia cell line, CHME-5 was infected in vitro with either a HIV-luciferase pseudovirus (HIV-Luc) or wild type HIV-NLAD8 in absence or presence of the major psychoactive component of marijuana tetrahydrocannabinol(THC). Cytokine/chemokine responses were monitored. Microglia infected with HIV-Luc and exposed to THC exhibited increased viral replication compared to untreated cells. But, microglia infected with wild type HIV did not display significant modulation in HIV replication. Recent evidence demonstrated that HIV-infected glia cells release the HIV accessory protein Negative factor (Nef) in exosomes and these Nef+ exosomes (exNef) may play a role in HIV neuropathogenesis. To examine the effect of THC on exNef release and modulation of

microglia immune function, nef-transfected microglia were treated with THC for 48 hours. Supernatants collected, exosomes separated, and analyzed via SDS/PAGE. THC modulated protein content and exNef levels. THC reduced inflammatory cytokines demonstrated by cytokine array. Impact of THC on monocyte migration across an in vitro model of the blood-brain-barrier (BBB) alone or with exNef was examined. THC reduced monocyte migration across the BBB suggesting that THC inhibited exNef-mediated immunopathology in the brain.

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METH-induced, TAAR1-associated CREB signaling serves as a master regulator for astrocyte EAAT-2

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Methamphetamine (METH) abuse accelerates the onset and severity of HIV-associated neurocognitive disorders (HAND) and astrocyte-mediated excitotoxicity. METH targets several receptors, particularly astrocyte trace amine associated receptor 1 (TAAR1), as we have previously reported. Molecular alterations of astrocyte TAAR1 correspond to changes in astrocyte excitatory amino acid transporter-2 (EAAT-2) levels and function; however, the signaling pathways downstream of METH-induced TAAR1 activation remain unclear. Astrocyte EAAT-2 is tightly regulated at the transcriptional and translational levels by cAMP and calcium, yet METH-mediated increases in these second messengers have not been shown to directly modulate astrocyte EAAT-2. Furthermore, HIV-1 relevant stimuli and IL-1β, increase TAAR1 and may exacerbate METH-mediated excitotoxicity via MAPK/ERK and NF-κB. We propose CREB activation serves as a master regulator of astrocyte EAAT-2. To investigate the temporal order of CREB activation we utilized genetically encoded calcium indicators, or GCaMPs, to visualize and quantify METH-induced calcium signaling. RNA interference targeting PKA and NF-κB subunit p65, in addition to PKA and MAPK/ERK specific inhibitors support their involvement in astrocyte EAAT-2 regulation. Furthermore, we investigated CREB phosphorylation at serine 133/142, the co-activator and co-repressor forms, respectively, following METH-induced activation. Overall, this work identifies critical signaling pathways and therapeutic targets for astrocyte EAAT-2 recovery.

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Gluthatione reduces lysosomal disruption in HIV infected macrophages

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In HIV infection and neurocognitive disorders (HAND), oxidative stress is an important hallmark. HIV infection and oxidative stress promotes the secretion of the lysosomal protease cathepsin B in monocyte-derived macrophages (MDM), which induces neurotoxicity. HIV-1 infection also disrupt the interaction between cathepsin B and its inhibitors, cystatins B and C. Therefore we hypothesized that treatment of macrophages with glutathione (GSH) will restore the interactions of cathepsin B with its inhibitors and the integrity of the lysosomes, reducing the secretion of cathepsin B. HIV-1 infected MDM, were treated with GSH at one day post-infection (dpi), and disruption of lysosomes was assessed using LysoPainter dye. Cathepsin B/cystatin B interaction was determined at 12dpi by proximity ligation assay. We found that lysosomal disruption and cathepsin B/cystatin B interaction were restored in HIV-1infected MDM after GSH treatment. Interestingly when Efavirenz, a non-nucleoside reverse transcriptase inhibitor was added to MDM, the levels HIV infection decreased significantly and the lysosomal disruption was also restored, suggesting that HIV infection is damaging the lysosomes and inducing cathepsin B secretion. Combining GSH with antiviral therapy, may reduce HIV replication, oxidative stress and macrophage dysfunction. Therefore we conclude that treatment of macrophages with GSH restores the interactions of cathepsin B with its inhibitors and the integrity of the lysosomes.

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HIV-1 Tg rat: Selective alterations in locomotor activity in the open field vs. voluntary wheel running

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HIV-1 Transgenic (Tg) rats have altered locomotor activity compared to control rats. In the present study, we compared adult ovariectomized female HIV-1 Tg animals (n=21) to F344 controls (n=26) in open field locomotor activity and voluntary wheel-running tasks. The animals were housed in pairs under a 12:12 light/dark cycle. Locomotor activity was assessed using open field locomotor chambers via infrared photocells for 60 minutes during the light phase of the diurnal cycle. Movements were defined as either gross, fine, or rearing. All measures of activity were significantly decreased ($p<0.001$) in the HIV-1 Tg animals compared to F344 control rats. Voluntary running was measured with 34cm-diameter running wheels for 60 minutes during the light phase of the diurnal cycle. There were no significant differences between HIV-1 Tg and F344 control rats in voluntary wheel running. Furthermore, the reduced activity of the HIV-1 Tg animals, which was noted in the open field measures, was not apparent in voluntary wheel running. Within-animal comparisons of activity measures and wheel-running showed that the open field activity data were not correlated with voluntary wheel running data ($r2s<0.025$). Collectively, these data indicate that the task chosen for assessment of motor behavior may be a critical determinant of sensitivity to detection of the effects of the HIV-1 transgene. We hypothesize the selective alterations in the motor behavior of the HIV-1 Tg rat may be more reflective of exploratory behavior rather than motivation per se.

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Astrocytic HIV-1 Nef expression causes learning impairment and inflammation

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Success in managing HIV replication by combination antiretroviral therapy (cART) emphasizes the need to address long term morbidities such as HIV associated neurocognitive disorders. HIV-1 Nef is an early viral protein with demonstrable neurotoxicity. Nef is of particular interest as it is produced by astrocytes which are infected but do not support robust viral replication which is the primary target of cART. We investigated the effect of astrocytic expression of Nef on learning and inflammatory responses in a rat model. Primary rat astrocytes expressing Nef or GFP (control) were infused in the hippocampus of male Sprague-Dawley rats; fluorescently labeled monocytes were delivered via tail vein. Learning was assessed by Morris water maze (5 and 10 days after infusion).Various tissues and blood samples were collected for histological and immunological analyses. GFP controls learned normally showing reduced latency across trials ($p<0.01$) and increased probe trial exploration ($p<0.01$); Nef exposed rats showed spatial learning impairment in these tests. Nef expression increased number of Peyer's patches in the ileum, CCL2 levels in serum, colonic damage ($p<0.01$) and macrophage infiltration in the liver ($p<0.01$). Claudin-5, a junction protein, showed reduced expression in the lungs. We have shown that Nef expression by astrocytes in the brain causes learning impairment which is prolonged across ten days and promotes systemic inflammation and pathology. This suggests viral protein expression from non-productively infected cells may contribute to HIV pathogenesis in the cART era.

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Enhanced Autophagy Of Pulmonary Endothelial Cells On Exposure To HIV-Tat And Morphine: Role In HRPAH Associated Pulmonary Vascular Remodeling

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Intravenous drug use is major risk factor for HIV related pulmonary arterial hypertension. Our ex-vivo analysis of SIV+/- morphine exposed macaque lungs and in-vitro studies on morphine and HIV protein(s) exposure showed enhanced vascular remodeling and increased apoptosis followed by aggravated proliferation of pulmonary endothelial cells (Am J Respir Crit Care Med. 2012;185(11):1235-43). We hypothesize

that the switching of cells from apoptotic to hyperproliferative state on combined HIV-Tat and morphine exposure may be due to exacerbation of autophagy. On treating human pulmonary microvascular endothelial cells (HPMEC) with morphine and Tat, we observed significantly higher increase in autophagy markers by western blot at 3h to 24h compared to morphine or Tat mono-treatment. TEM and immunofluorescence showed remarkably higher autophagosome formation in 24h morphine and Tat exposed HPMEC compared to mono-treatments. Autophagic flux was indicated by further increase in LC3BII and p62 in cells pre-treated with bafilomycin-A followed by 24h of morphine-Tat exposure. Autophagy inhibition increased morphine-Tat mediated apoptosis and decreased HPMEC proliferation, whereas autophagy stimulation significantly reduced apoptosis and increased proliferation. These effects were higher in cells treated with morphine+Tat compared to mono-treatments. Hence, we conclude that morphine and Tat synergistically induce autophagy of HPMEC that may lead to increased severity of angio-proliferative remodeling of pulmonary vasculature in SIV/HIV-infection in presence of opioids.

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Longitudinal surveillance sequencing reveals variability despite undetectable viral loads

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While highly active anti-retroviral therapy (HAART) has proven an effective way to control viral loads within the peripheral blood compartment, it is less able to control replication in reservoirs such as the brain. The Drexel Medicine CNS AIDS Research and Eradication Study (CARES) cohort has tracked more than 500 patients longitudinally with a subset of patients that have been followed for more the 10 well-

controlled visits spanning more than 7 years. The LTR and Tat exon 1 region of these patients were examined using the Bayesian Evolutionary Analysis Sampling Trees (BEAST) program to examine underlying phylogenetic progression of their viral population. Patients were found to have a basal level genetic drift despite undetectable viral loads. As a result of this analysis the detected drift ranged from 0.2-2 bases per 100 nucleotides per year and was unique for each patient. Phylogenetic analysis of these patients revealed that sequence variation between sequential visits may be due in part to differential activation of latent proviral DNA quasispecies and/or low level viral replication in various reservoirs that may occur even in well-controlled patient populations over prolonged time and ultimately detected in the peripheral blood compartment. Further analysis with viral deep sequencing across longitudinal samples across the entire viral genome is expected to reveal a more exacting view of the rate and overall nature of genetic drift even in well-controlled HIV-1-infected patients.

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Methamphetamine decreases voltage-gated potassium currents in primary human fetal astrocytes

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Methamphetamine (Meth) is a potent and commonly abused psychostimulant. Exposure to Meth induces decreased neuronal activity in the medial prefrontal cortex and nucleus accumbens which are implicated in addiction; but it is unknown if such a decrease results from alterations in synaptic/intrinsic excitability of neurons or changes in extracellular environment (e.g., glutamate and K+ levels) mediated by surrounding astrocytes. To fill this knowledge gap, we assessed the effects of Meth on functional activity of certain voltage-gated ion channels in the cell membrane of primary human fetal astrocytes (HFA) using whole-cell voltage-clamp recording. We found that HFA displayed a large out-flowing voltage-gated K+ current (VGKC, a characteristic of immature or reactive astrocytes), while voltage-gated Ca2+ currents were not seen.

Further, exposure of HFA to Meth (300 μM, 3-6 hrs) induced a significant depolarization of the resting membrane potential (RMP) and reduction of VGKC in the range of +70 to 100 mV in comparison to untreated HFAs (both $p<0.05$). Our findings demonstrate that Meth disturbs HFA activity by altering RMP and VGKC. Because a Meth-induced decrease of K+ out-flow from astrocytes can consequentially reduce local extracellular K+ levels, such a reduction could ultimately lead to a decreased excitability in neurons surrounded by these astrocytes.

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Marijuana promotes HIV replication in glia cells but inhibits HIV-exNef associated neuropathology

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Marijuana has been shown to inhibit HIV-associated immunopathology. However, the effect of marijuana exposure on HIV replication in the brain and neuropathology is unclear. Here we perform a series of in vitro studies using a mixed-cell model of the blood-brain-barrier (BBB) and a single cycle HIV-luciferase pseudovirus (HIV-Luc) to investigate the impact of marijuana on HIV replication in astrocytes. Astrocytes were infected with HIV-Luc in absence or presence of the major psychoactive component of marijuana tetrahydrocannabinol (THC). Astrocytes exposed to THC exhibited increased viral replication compared to untreated cells. Recent evidence demonstrated that HIV-infected glia cells release the HIV accessory protein Negative factor (Nef) in exosomes and these Nef+ exosomes (exNef) impair the BBB. To examine the effect of THC on exNef, nef-transfected microglia and astrocytes were treated with THC for 48 hours. Supernatants were collected, exosomes separated, and analyzed via SDS/PAGE. THC modulated exNef levels in nef-transfected microglia and astrocytes. In the mixed-cell BBB, THC alone had no significant effect on the integrity of the BBB as measured by transendothelial resistance (TEER) while exNef reduced BBB TEER values. Combination of THC with exNef restored TEER values to that of control. The same result was observed for FITC-Dextran transport assays measuring BBB permeability

suggesting that THC inhibits exNef function. Taken together, these results show that THC up-regulates HIV replication in glia cells while inhibiting exNef-mediated neuropathology.

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Using the lip scarification model to study HSV-1 replication kinetics and the CD8+ T-cell response

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Herpes simplex virus type 1 (HSV-1) is a human pathogen that replicates in oral and ocular tissues during primary infection before establishing a lifelong latent infection within the trigeminal ganglion (TG). The immune system is critical for controlling the initial infection and for establishing and maintaining viral latency. HSV-1 infection in humans occurs with clinical symptoms that include asymptomatic disease to lesions following infections of the lip and eye. There is a well-established ocular infection model in the laboratory mouse which reproduces primary infection and latency observed in humans. The majority of primary human infections occur within the lip and oral cavity in contrast to the ocular tissue. We used the lip scarification model to define the kinetics of HSV-1 replication and CD8+ T cell response during primary infection, TG invasion, and establishment of viral latency. Three-month-old mice were inoculated with HSV-1 and tissue was collected at 7 time points up to day 60 post-infection for detection of infectious virus and responding CD8+ T cells. High virus titers were detected in the lip at early time points that resolved after 8 days of infection. The virus infiltrated the TG during primary infection and latency was established by 30 days post-infection. CD8+ T cells were observed infiltrating the TG 8 days post-infection and were present 60 days post-infection. These results demonstrate that the lip scarification model can be used to study viral kinetics and the role of CD8+ T cells in controlling viral infection of the TG and the periphery.

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Cannabidiol-induced myeloid-derived suppressor cells ameliorate experimental autoimmune encephalomyelitis through microRNA regulation

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Multiple Sclerosis (MS) is a chronic neurodegenerative autoimmune disease that affects ~400,000 people in the US. Current therapies have harsh side effects and there is a need for more effective treatment modalities. The use of cannabinoids for MS has been recently explored. However, the mechanism of action has not been completely elucidated. We used experimental autoimmune encephalomyelitis (EAE), a murine model of MS, to explore the anti-inflammatory role of cannabidiol (CBD). EAE disease paradigms were consistently reduced with CBD treatment, shown by a significant reduction in clinical scores of paralysis and decrease in cellular infiltration, marked improvement of CNS tissue integrity, and reduced demyelination. In addition, CBD treatment led to a reduction in the percentage and absolute number of T cells particularly the CD4+ T cells infiltrating the CNS (spinal cord and brain), which were significantly increased in the untreated EAE mice. Interestingly, CBD treatment led to a profound increase in MDSC induction in the spleen, CNS, and peritoneal cavity of CBD treated EAE mice as compared to the untreated EAE controls. Moreover, adoptive transfer of CBD-induced MDSCs ameliorated EAE and MDSC depletion (anti-Gr1) reversed the beneficial effects of CBD treatment. MicroRNA microarray analysis revealed an altered miRNA profile in peripheral CD4+ T cells following CBD post-treatment of EAE mice. Together, these studies demonstrate that CBD treatment may ameliorate EAE via the induction of MDSCs and miRNA regulation which suppress the aberrant autoimmune response.

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PCB153-induced loss of occludin and disruption of endothelial barrier through lipid raft-mediated PP2A and MMP-2

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Occludin is an essential integral transmembrane protein regulating tight junction (TJ) integrity in brain endothelial cells. Phosphorylation of occludin is associated with its localization to TJ sites and incorporation into intact TJ assembly. Exposure of human brain endothelial cells to 2,2',4,4',5,5'-hexachlorobiphenyl (PCB153), a representative of ortho-substituted PCB, induced a displacement of occludin from detergent-resistant membrane (DRM)/lipid raft fractions in which intact TJs are assembled. Inhibitiors against protein phosphatase 2A (PP2A) activity, okadaic acid or fostriecin markedly protected against PCB153-induced displacement of occludin and disruption of endothelial integrity. The implication of lipid rafts and PP2A signaling in these processes was further defined by co-immunoprecipitation of occludin with PP2A and caveolin-1. In addition, occludin levels may be regulated by activation of metalloproteinases (MMPs). Indeed, a significant MMP-2 activity was observed in lipid rafts and its inhibition protected against PCB153-induced loss of occludin and increased endothelial permeability. Overall, these results indicate that lipid raft-associated processes, such as PP2A and MMP-2 activities, participate in PCB153-induced displacement and degradation of occludin from TJs. This study contributes to a better understanding of the mechanisms leading to loss of endothelial barrier function in response to exposure to environmental pollutants, such as ortho-substituted PCBs.

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Inflammatory cytokine effects on neurotransmitters, neurocircuits, and behavior in depression

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Recent evidence suggests a cause and effect relationship between inflammation and the development of depressive symptoms in both medically ill and medically healthy individuals. Biomarkers of inflammation, such as inflammatory cytokines and acute-phase reactants, are reliably elevated in a significant proportion of depressed patients. In addition, administration of cytokines or inflammatory stimuli is associated with development of depressive symptoms in laboratory animals and humans. Furthermore, inhibition of inflammatory cytokines improves depressive symptoms in patients with inflammatory disorders and in medically healthy patients with major depression. Therefore, behavioral co-morbidities that are common complications of cancer and HIV may be related, in part, to the impact of inflammation and associated release of inflammatory cytokines on the brain. Recent neuroimaging data from our group and others demonstrate that cytokines and other inflammatory stimuli affect relevant basal ganglia nuclei to lead to depressive symptoms of anhedonia and psychomotor slowing. Similar studies have also shown that inflammation affects brain regions in the frontal cortex, such as the anterior cingulate cortex, which may be relevant to symptoms of anxiety. These inflammation-related changes in neural activation and metabolism have been linked to inflammatory cytokine effects on the dopamine and glutamate neurotransmitter systems, which may serve as targets for novel therapeutic strategies to reverse inflammation effects on the brain.

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The Role of P2X7R Antagonism in METH-induced CPP

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Methamphetamine (METH) is a highly abused psychostimulant that is associated with neurotoxicity to dopaminergic neurons with no approved treatments. METH blocks the

activity of the dopamine transporter (DAT), inhibits vesicular monoamine transporter-2, and reverses transport of dopamine (DA) via DAT leading to aberrant DA release and neurotoxicity, which contribute to behavioral and cognitive impairments associated with METH abuse. Recently the use of P2X7-competitive antagonist A438079 to prevent DA depletion in a model of neurodegeneration implicates purinergic signaling in DA-mediated neurotoxicity. Our prior studies in an escalating-dose METH animal model demonstrated increased P2X7R expression in striatum and identified an important role of P2X7R in METH-induced microglial activation responses. Subcutaneous (SQ) administration of 5mg/kg METH to male C57Bl/6 mice (n=6/group) produced a 400s shift ($p<0.0031$) in conditioned place preference (CPP). In ongoing studies we test the hypotheses that pretreatment with A438079 could significantly decrease METH-induced CPP. To determine its effect on expression of METH-induced CPP, A438079 is intraperitoneally administered 1H before the post-test. To demonstrate that P2X7 antagonism blocks METH-induced development of CPP, male C57Bl/6 mice (n=8/group) are pretreated with A438079 1H before SQ administration of METH (5mg/kg) or saline and confined to non-preferred or preferred compartments, respectively, over 13 days. Taken together, these ongoing experiments examine the role of P2X7R in blocking METH-induced reinforcement.

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Synaptodendritic injury in HAND: role of IL1-beta mediated induction of the protein ferritin heavy chain in cortical neurons

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Synaptodendritic injury, including reduction in dendritic spine density, is a hallmark of HIV-associated neurocognitive disorders (HAND). Both host (e.g. inflammatory cytokines) and viral (e.g. HIV-1 gp120) factors contribute to neuronal damage but the molecular mechanisms are only partially understood. Published work from the laboratory demonstrated that ferritin heavy chain (FHC) disrupts homeostatic chemokine signaling in neurons, including CXCL12's ability to increase dendritic spine density, and suggested a role for FHC in the cognitive impairment of HIV patients. We also recently found that the gp120-evoked release of IL1-beta from glial cells leads to

induction of FHC in cultured neurons. To further evaluate the consequences of FHC changes induced by viral proteins in vivo, we used two rodent models of HAND: gp120-treated and HIV-Tg rats. Briefly, in these animals, we observe reductions in both dendritic spine density and basal dendrite branching in layer II/III pyramidal neurons of the medial prefrontal cortex. Importantly, dendritic spine density statistically correlated with reversal learning, a measure of behavioral flexibility found to be impaired in gp120-treated rats. Furthermore, we found elevated levels of FHC in brain lysates of HIV-Tg rats. Together with our previous findings, these studies reinforce FHC's role in cognitive impairment of HAND patients, point to IL1-beta as a valid therapeutic target for HAND, and have major implications for drug abuse, as neuronal FHC levels in drug users are altered regardless of HIV status.

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C/EBPβ expression in the brain during HIV infection: Implications for neuronal gene expression

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Despite monumental advances in antiretroviral therapy, HIV-associated neurocognitive disorders (HAND) prevalence remains high and is expected to increase as the HIV infected population ages. C/EBPβ is a transcription factor that regulates gene expression involved in inflammation, differentiation, HIV replication and autophagy. Despite much research, details are still lacking in regards to transcriptional regulation of gene expression in the brain during HIVE. Here, we identified C/EBPβ expression levels in postmortem brain samples from a cohort of well-characterized control and HIV+ patients with and without HIV encephalitis (E) and also with increasing HAND severity. Detailed clinical and neuropathological data showed the HIVE and HIV-associated dementia (HAD) patients had higher viral load (VL), increased neuroinflammation and elevated neurodegeneration compared to HIV+ patients, and HIVE postmortem brain tissues showed the most severe neurodegenerative pathology. C/EBPβ protein and mRNA levels were higher in post-mortem tissues from HIV+ brains compared to control, and levels were highest in post-mortem tissues from HIVE and HAD brains. C/EBPβ was detectable in microglia, astrocytes and neuronal cells by immunohistochemistry in

all patients, but expression was predominantly astroglial in HIVE brains. These data suggest that C/EBPβ is active in astrocytes during HIVE and controls transcription of genes in response to HIV infection of the CNS. Hence, C/EBPβ may provide a therapeutic target useful to ameliorate many neurodegenerative diseases, including HAND.

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Interferon-gamma producing immune cells play a role during neonatal central nervous system infections

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Viral infections in the central nervous system (CNS) are associated with devastating neurological consequences, particularly in newborns. Despite mounting innate and adaptive immune responses, neonates are often unable to control viruses in the brain and suffer extensive neuronal loss, potentially due to deficits in anti-viral cytokines. To study the neonatal immune response to viral CNS infections, our laboratory uses a transgenic mouse model (NSE-CD46) of neuron-restricted measles virus (MV) infection. NSE-CD46 mice express the human isoform of CD46, a MV receptor, under the control of the neuron specific enolase (NSE) promoter, allowing for infection only in CNS neurons. Adult NSE-CD46 mice clear MV from the CNS in an interferon gamma (IFNg) and T cell dependent manner. In contrast, neonatal NSE-CD46 mice succumb, with 100% mortality at 15 days post infection (dpi). Neonatal mice lacking IFNg (CD46+/IFNg-KO) succumb more rapidly than NSE-CD46+ neonates (100% mortality, 10 dpi) despite higher T cell infiltration and similar natural killer cell infiltration in the CNS. CD46+/RAG2-KO neonates, which lack T- and B-cells, show reduced mortality in comparison to other CD46+ genotypes and have lower MV RNA. We hypothesize that an inadequate cytokine response in neonates may contribute to a lack of viral control and CNS pathology. Current experiments aim to define anti-viral cytokine profiles in the brains of infected neonatal and adult mice and to determine mechanisms of neuronal loss in neonatal brains. This work is funded by NINDS Grant 1R15NS087606-01A1.

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Methamphetamine synergistically interacts with HIV-1 Vpr in the induction of IL-6 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. Furthermore, methamphetamine has been not only shown to accelerate the virus replication in in-vitro and in-vivo studies that would lead to increased production of viral proteins, but it has also been shown to cause expression of proinflammatory cytokines. Proinflammatory cytokines such as IL-6 have been shown to produce neurotoxic effects in the brain. We have shown that HIV-1 Vpr induces IL-6 in astrocytes, however it is not known if methamphetamine will have additive effects with HIV-1 Vpr in the production of IL-6. To address this question, we treated SVGA astrocytes with various concentrations of methamphetamine for 3 days and transfected them with either mock plasmid or a plasmid encoding HIV-1 Vpr. We observed that methamphetamine (10µM, 100µM and 500µM) caused dose dependent synergistic induction of IL-6 by HIV-1 Vpr. Further, we explored the involvement of NF-κB using chemical inhibitor and siRNA's. Our results suggest the involvement of NF-κB in methamphetamine and HIV-1 Vpr mediated increased production of IL-6. Additional set of experiments suggest that PI3K/Akt might be upstream pathway responsible for NF-κB activation and synergistic induction of IL-6 by methamphetamine and HIV-1 Vpr. 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-1 associated neurological disorders (HAND).

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ACTIVATION OF DOPAMINE RECEPTORS INCREASES HIV ENTRY INTO MACROPHAGES BY ALTERING CALCIUM RELEASE

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Macrophages and microglia are the primary cell types infected with HIV in the CNS, as well as the primary source of virus in this tissue. In brains of drug abusers, these cells are exposed to the neurotransmitter dopamine, which is elevated by the use of all addictive substances including methamphetamine, heroin, alcohol, and many prescription drugs. Our previous research has shown that this increase in CNS dopamine could play a significant role in the development of HIV-associated neuropathogenesis. Dopamine increases HIV replication in macrophages and modulates their production of inflammatory cytokines. The increase in replication is mediated by enhancing the susceptibility of macrophages to viral entry mediated through the CCR5 entry pathway. This effect requires activation of either D1-like or D2-like dopamine receptors, and occurs at dopamine concentrations above 10-8M. The involvement of both dopamine receptor subtypes indicates a common signaling pathway. Our new data suggest that this pathway involves dopamine receptor-mediated potentiation of calcium flux, as blocking calcium release interferes with the effects of dopamine on entry. Thus, despite different mechanisms of action, the use of all types of illicit drugs could accelerate the development of neuroinflammation and HAND through the effects of elevated dopamine infection of CNS macrophages.

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MECHANISMS OF ANGIOTENSIN II-INDUCED OXIDATIVE STRESS IN HUMAN NEURONAL CELLS

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Neuronal oxidative stress (OS) and injury induced by Angiotensin II (AngII) has been implicated in the pathogenesis of several neurodegenerative disorders. However, the

precise mechanism by which AngII mediates these effects in neuronal cells remains unclear. Here, we investigated if neuronal OS and apoptosis induced by AngII were mediated through its AT1 or AT2 receptors and the enzyme xanthine oxidase (XO). Human SH-SY5Y neuronal cells were cultured in the presence of AngII (100-1000nM), AngII plus Losartan (1uM; AT1 blocker), or AngII plus PD123177 (1uM; AT2 blocker) for 24 hours. Neuronal OS and apoptosis were analyzed by flow cytometry (FC) using a ROS reagent and AnnexinV/PI, respectively. Cells were also exposed to AngII plus Allopurinol (10uM; XO inhibitor) and OS was analyzed by FC. Our results indicated that: (1) Neuronal OS significantly (p=0.011) increased after incubation with AngII at 600nM; (2) Losartan was not able to antagonize the effects of AngII in OS, however PD123177 significantly decreased (p=0.037) the levels of ROS; (3) The percentage of apoptotic cells significantly increased (p=0.019) after AngII treatment; (4) Allopurinol significantly decreased (p=0.005) ROS levels in the presence of AngII, however these levels were higher when compared to cells exposed to AngII plus PD123177. Our findings support that the detrimental effects of AngII in neuronal cells are mediated by the AT2 receptor, and at least partially, through the activation of XO. Our results may help to clarify the benefits of AngII Receptor Blockers in neurodegenerative disorders.

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HTLV-1 infection and T-cell exhaustion in Rag1-/-γc-/- (RAG1) mouse model

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Animal models have been widely employed to study HTLV-1 transmission, pathogenesis, and treatment. To better understand the immuno- and neuropathogenesis of HTLV-1 in an in vivo system we employ here the Balb/c-Balb/c-

Rag1 −/− γc −/− or Rag1 (both neonates and adults) as well as Bone marrow-Liver-Thymic (BLT) mouse models for engraftment of human CD34+ hematopoietic stem cells. Flow cytometry and histological analyses revealed reconstitution of Rag1 mice with human immune cells, including macrophages, dendritic cells, T cells and B cells. Proviral load (PVL) was determined in the peripheral blood and spleen of Rag1 and BLT mice by the droplet digital PCR method. Within blood, PVL was detected as early as 2 wks post-infection (wpi), which was high compared to PVL levels in the spleen at its peak. The viral protein Tax showed peak expression at 14 wpi in Rag1 mice with continued expression until 16 weeks. The PVL and Tax expression was considerably higher in the adult Rag1 mice as compared to the neonates with less than 20% PVL in the peripheral blood, brain, and liver in the neonates. The inhibitory receptor (PD-1) and its ligand (PDL-1) have been associated with T-cell dysfunctions in HTLV-infected patients (ATL and HAM/TSP), thus we assessed PD-1 expression on human T cells from the spleen of HTLV-1-infected mice. At 5 wpi, we found an increased percentage of CD4+ and CD8+ T cells expressing PD-1 upon infection in peripheral blood compared to control. This data represent the first attempt to establish HTLV-1 pathogenesis in the context of RAG1 and BLT mice.

Devising a glycoantigen specific DC immunotherapy to develop an efficient anti-tumor response in the hypoxic environment of glioblastoma.

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Glioblastoma (GBM) is the most malignant type of brain tumor, its therapy hindered by the hypoxic microenvironment that promotes tumor resistance and inhibits immune cell

function. We propose combining dendritic cell (DC) immunotherapy with hypoxia reversal to eradicate GBM. HIF-1α, a transcription factor that stimulates genes promoting angiogenesis and tumor growth, was expressed in both GBM cells and DCs under hypoxia. Hypoxia also downregulated expression of HLA-DR, CD86 and HLA-ABC on DCs affecting their antigen processing and presentation capability in addition to reducing the generation of pro-inflammatory cytokines such as TNF-α and IL-6. In vivo imaging revealed fluorescently labeled DCs migrating away from site of injection into spleen and mediastinal lymph nodes of mice with GBM indicating chemoattraction of DCs to areas of inflammation post hypoxia reversal using using antisense HIF-1Î± plasmid. In order to identify tumor antigens we used glycan analysis wherein GlcNAc was overexpressed in hypoxic GBM cells. We then enriched GlcNAc expressing glycoantigens and identified 46 glycopeptides derived from 33 glycoproteins. Amongst them Lamb1, SerpinH1, CD63 and others are associated with tumor survival, progression, invasion, immune evasion and therapy resistance. Lectins expressed on DCs complimentary to these glycans will be identified to devise a DC based vaccine, which in conjunction with hypoxia reversal will augment the efficacy of existing therapies against GBM.

Potentiating dendritic cells to target hypoxic environment of brain tumor

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Glioblastoma (GBM) is the most malignant type of brain tumor, its therapy hindered by the hypoxic microenvironment that promotes tumor resistance and inhibits immune cell function. F98 and U87 glioma cells show up-regulation of HIF-1α, a transcription factor that stimulates genes that promote angiogenesis and tumor growth. We also confirm

that hypoxia increases HIF-1Î± expression in dendritic cells (DCs), but downregulates the expression of HLA-DR, CD86 and HLA-ABC affecting their antigen processing and presentation capability in addition to reducing the generation of pro-inflammatory cytokines such as TNF- α and IL-6. Using antisense HIF-1Î± plasmid we then reversed the effect of hypoxia on DC functionality. In vivo imaging revealed fluorescently labeled DCs migrating away from site of injection into spleen and mediastinal lymph nodes of mice with GBM indicating chemoattraction of DCs to areas of inflammation. In order to produce tumor-specific DC response we identified GlcNAc, over expressed in hypoxic GBM cells, using glycan analysis to select a potential tumor antigen. We then enriched for GlcNAc expressing glycoantigens identifying 46 glycopeptides derived from 33 glycoproteins with aberrant GlcNAc glycosylation. Lamb1, SerpinH1, CD63 and other glycoproteins identified are associated with tumor survival, progression, invasion, immune evasion and therapy resistance. Lectins expressed on DCs complimentary to these glycans will be identified to devise a DC based vaccine, which in conjunction with hypoxia reversal will augment the efficacy of existing therapies against GBM.

HSV vector-mediated GAD67 suppresses neuropathic pain induced by HIV gp120 combined with ddC through reducing ROS in rats

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Despite of extensive research of HIV-associated neuropathic pain, the exact neuropathological mechanisms remain unknown, hindering our ability to develop effective treatments. Loss of gamma-aminobutyric acid (GABA) GABAergic inhibitory mechanisms has been reported after peripheral nerve injury. In this study, we tested the hypothesis that HIV gp120 combined with anti-retroviral therapy reduce spinal GABAergic inhibitory tone, and that restoration of GABAergic inhibitory tone will reduce HIV-related neuropathic pain in rat. The application of recombinant HIV gp120 into the sciatic nerve plus systemic ddC (one antiretroviral drug) induced mechanical allodynia. The hind paws of rats were inoculated with replication-defective herpes simplex virus (HSV) vectors genetically encoding gad1 gene to express glutamic acid decarboxylase

67 (GAD67), an enzyme that catalyzes the decarboxylation of glutamate to GABA. In the gp120 with ddC-induced neuropathic pain model, GAD67 mediated by the HSV vector caused an elevation of mechanical threshold. The anti-allodynic effect of the single HSV vector inoculation expressing GAD67 lasted more than 28 days. The HSV vectors expressing GAD67 reversed the lowered GABA-IR in the spinal dorsal horn in the neuropathic rats. HSV vectors expressing GAD67 in the neuropathic rats reduced the signals of mitochondrial superoxide in the spinal dorsal horn. Based on these results above, we suggest that GAD67 mediated by HSV vectors decreases HIV-related neuropathic pain through the suppression of mitochondrial ROS in the spinal cord.

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Inflammation-associated microRNAs increase in hippocampal neurons in a controlled cortical impact mouse model of traumatic brain injury

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Inflammation contributes to neuronal cell death and dysfunction following traumatic brain injury (TBI). MicroRNAs (miRNAs) regulate the magnitude and duration of inflammation in many cell and tissue types through post-transcriptional regulation of target transcripts. We hypothesized that inflammation-associated miRNAs would increase in a controlled cortical impact (CCI) model of TBI. We found that the expression of all of the inflammation-associated miRNAs examined (miR-223, miR-155, and miR-21) increased in the injured hippocampus relative to the contralateral hippocampus following moderate CCI (0.5 mm depth) in mice. The level of mature miRNAs in the injured and uninjured hippocampus was determined by qPCR at 1, 3, 7, and 14 days post-CCI, with animals exposed only to a craniotomy as controls. Levels of miR-223 increased acutely, with levels only elevated at 1 day post-injury. The expression of miR-155 was more sustained with increases at 1, 3 and 7 days post-injury. In contrast, increased miR-21 expression was not observed until 14 days post-injury. In situ hybridization for miR-223, miR-155 and miR-21 revealed that the observed increases in inflammation-associated miRNAs were primarily localized to neurons.

These findings suggest that inflammation-associated miRNAs may play a role in the neuronal response to TBI.

Effects of CB2R/GPR55 agonist treatment on neural progenitor cell immune responses to chronic inflammation

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New neurons are produced by neural progenitor cells (NPCs) within the adult hippocampus. Numerous diseases including major depressive disorder (MDD) and HIV-1 associated neurocognitive disorder (HAND) are associated with decreased rates of adult neurogenesis. A hallmark of these conditions is a chronic release of neuroinflammatory mediators by activated resident glia. Recently, cannabinoid receptor 2 (CB2R) has been reported to modulate regulation of neuroimmune responses and is a potential therapeutic target. In the present study we examined human NPCs exposed to HIV-1 and inflammatory cytokines to assess inflammation-caused effects on NPC proliferation and differentiation and the ability for selective CB2R agonists and candidate cannabinoid receptor GPR55 agonists to attenuate NPC injury. Protective effects of CB2 and GPR55 agonists were assessed after treating an in vitro non-proliferating phenotype of human NPCs with inflammatory cytokines and HIV-1. Expression of inflammation associated receptors, CB2R and GPR55 was determined via qPCR and FACS. NPC proliferation was evaluated via BrdU incorporation. NPC differentiation and neurogenesis was determined via immunocytochemical analysis of NSC markers (Nestin, Sox2, DCX, NeuN, TUJ-1). Results showed an alteration of receptor expression on NPCs after exposure to inflammatory mediators. CB2R/GPR55 treatment aided in regulating inflammatory responses of NPCs in vitro. Proliferation rate and total numbers of immature neurons were significantly reduced in an animal model of low-dose, systemic chronic inflammation.

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Phytoestrogen Equol Prevents HIV-1+Cocaine Induced Synaptodendritic Injury via Estrogen Receptor Beta Mediated Mechanism

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Synaptic loss is closely correlated with the severity HAND. HIV-1 Tat and cocaine have also been known to cause neuronal death. Previous work in our lab has shown that estradiol and phytoestrogens prevent Tat-mediated neuronal apoptosis. Equol, a compound with S- and R- enantiomers, is a metabolite of the phytoestrogen daidzien and is more potent than daidzien. Pre-treatment of estrogen receptor antagonist tamoxifen (100nM) was shown to abolish the synaptodendritic protection of both enantiomers of equol. This study determines which estrogen receptor S- and R-Equol act to prevent Tat and cocaine mediated synaptodendritic damage. Primary rat cortical cell cultures (21 days in vitro) were treated with Tat 1-86B (10nM) and cocaine (1.6μM). The two nuclear estrogen receptors, α and β, as well as the cellular membrane estrogen receptor, GPR-30, were tested by treating the cultures using specific antagonists for each receptor. F-actin rich structures (puncta), which encompass a variety of pre- and post-synaptic structures, were manually counted to quantify synaptodendritic damage. We found that Tat + cocaine significantly reduced the amount of F-actin puncta density. However, S-Equol (50nM) and R-Equol (50nM) pretreatment significantly prevented Tat + cocaine synaptodendritic injury. The neuroprotective effects of both enantiomers of equol were blocked by the β-estrogen receptor antagonist, PHTPP ($p<.05$). These results suggest that a new therapeutic target may be used to help prevent synaptodendritic damage caused by HIV-1 and cocaine.

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Acidic store-operated calcium entry---involvement of lysosome calcium and lysosome exocytosis

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Endolysosomes are intracellular ‘acidic’ calcium stores that contain high concentrations of readily releasable calcium. Although ‘acidic calcium stores’ are present functionally in neurons, little is known about how calcium is released from the acidic stores and how this release contributes to calcium signals both spatially and temporally. In primary cultured hippocampal neurons, we found that calcium released from acidic calcium stores triggered calcium influx across the plasma membrane, a phenomenon we termed ‘acidic store-operated calcium entry’ (aSOCE). Because so little is known about how acidic calcium stores communicate with plasma membrane calcium channels, we tested here the hypothesis that calcium released from acidic calcium stores increases calcium influx through plasma membrane N-type calcium channels using a variety of methods including calcium imaging, electrophysiology, surface protein labeling, immunoprecipitation, and RNA interference. Similar to classical thapsigargin-induced store-operated calcium entry (SOCE), calcium released from acidic calcium stores, as induced by either inhibition of vacuolar H+-ATPase with bafilomycin or selective disruption of endolysosome membranes with GPN, triggered a dramatic influx of extracellular calcium. This aSOCE was attenuated significantly by blocking N-type calcium channels. Consistent with our calcium imaging data, our electrophysiological data demonstrated that both bafilomycin and GPN increased voltage-gated calcium currents. Biochemically, we demonstrated that bafilomycin and GPN both increased cell surface expression of N-type calcium channel and LAMP1, a lysosome associated membrane protein that plays an important role in lysosomal exocytosis. Furthermore, we observed a physical linkage between LAMP1 and N-type calcium channels. In addition, knocking down either LAMP1 or N-type calcium channels with RNA interference attenuated bafilomycin- or GPN- induced aSOCE. Collectively, our findings suggest that exocytotic insertion of N-type calcium channels might underlie this novel process of aSOCE in neurons. Such findings could provide a new insight into the spatiotemporal complexity of calcium signals and fundamental calcium-dependent cellular processes in neurons. (Supported by grant 2P20 RR0017699 from the NCRR, a component of the NIH.)

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CPEB plays an important role in the HIV neuropathic pain state through spinal ROS and Wnt signaling

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Both HIV infection-related distal sensory polyneuropathy (DSP) and antiretroviral toxic neuropathies contribute HIV-related painful sensory neuropathies (HIV-SN). However, the exact molecular mechanisms of HIV-associated neuropathic pain are not fully elucidated. Cytoplasmic-element-binding (CPEB) protein is a sequence-specific RNA-binding protein that regulates cytoplasmic polyadenylation-induced translation involved in inflammation. Recent evidence demonstrates that ROS and Wnt signaling are involved in the HIV neuropathy state. Here, we examined if CPEB plays a role in the HIV-related neuropathic pain. The application of recombinant HIV-1 envelope protein gp120 into the sciatic nerve plus systemic ddC (one antiretroviral drug) induced mechanical allodynia. Intrathecal administration of antisense oligodeoxynucleotide (ODN) against the expression of CPEB reduced the mechanical allodynia. HIV gp120 with ddC increased mitochondrial superoxide and Wnt5a in the spinal dorsal horn. The knockdown of CPEB with antisense ODN revsersed the upregulation of spinal ROS and Wnt5a in the model of gp120 with ddC. Based on these results above, we suggest that spinal CPEB plays an important role in the HIV neuropathic pain state through spinal ROS or Wnt signaling, providing preclinical evidence for novel pharmacotherapy approach to patients with HIV-related pain.

The effect of antiretroviral medications on neurogenesis

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Both HIV infection and antiretroviral (ARV) medications have been associated with neuronal toxicity, which may contribute to HIV associated neurocognitive disorders (HAND). The prevalence of HAND is rising despite combination ARV therapy (cART) since infected patients have longer survival times. Past studies have demonstrated that HAND patients manifest fewer neural stem cells (NSCs) in the dentate gyrus (DG) of the hippocampus, an important center for memory and learning. In the healthy state, neurogenesis occurs at a basal level in the dentate gyrus throughout adulthood. These neurons are subsequently integrated into existing neuronal networks. Further these newly generated neurons are more susceptible to oxidative stress and associated cytokine stress pathways than their adjacent, more mature granule cell neurons. Since ARV medications have previously been associated with stress pathway activation, we examined their effect on neurogenesis in vitro with rat NSC's as well as in C57BL/6 mice. Here we report that that lopinavir (LPV) and efavirenz (EFV), but not zidovudine (AZT), promote a decreases in neurogenesis through activation of stress pathways ultimately resulting in BAX upregulation. This was in conjunction with lowered NSC ATP stores and high levels of reactive oxygen species (ROS) imparted by these ARVs. In sum, these phenomena could both promote or be the result of our observed increased p38 MAPK phosphorylation which in turn would promote increased BAX expression manifesting as reduced neurogenesis.

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Novel Antiplatelet Activity of Minocycline Involves Inhibition of p38 MAPK

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Platelets play an essential role in hemostasis and wound healing by facilitating thrombus formation at sites of injury. Recently, it has also been demonstrated that

platelets also mediate inflammation. Not surprisingly, platelet dysfunction is known to contribute to several inflammatory disorders. Several antiplatelet therapies exist such as aspirin, adenosine diphosphate (ADP) antagonists, glycoprotein IIb/IIIa (GPIIb/IIIa) inhibitors, and anticoagulants that dampen platelet activity at the risk of unwarranted, excessive bleeding. Thus, the development of drugs that reduce platelet inflammation without interfering with thrombus formation is of importance to combat platelet related disorders. We have shown here that the tetracycline antibiotic, minocycline, has antiplatelet activity as it reduces soluble CD40L and platelet factor 4 levels in the plasma of HIV-infected patients. Minocycline reduced activation of isolated platelets in the presence thrombin as measured by ELISA and flow cytometry. Platelet degranulation was reduced upon exposure to minocycline as shown by mepacrine retention and flow cytometry. Minocycline had no effect on platelet spreading, aggregation, and GPIIb/IIIa activation. Lastly, immunoblot analysis suggests that this antiplatelet activity is likely mediated by inhibition of p38 MAPK phosphorylation and loss of p38 activity. Ultimately, this work will not only provide a better understanding for platelet biology but also a novel repurposing of an antibiotic to combat dysfunctional platelet activation in several inflammatory disorders.

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Effects of Buprenorphine on CCL2 mediated HIV Infected and Uninfected CD14+CD16+ Monocyte Migration in the context of neuroAIDS

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HIV-1 enters the CNS within two weeks after peripheral infection and results in chronic neuroinflammation that leads to HIV associated neurocognitive disorders (HAND) in more than 50% of infected people. HIV enters the CNS by transmigration of infected monocytes across the blood brain barrier. Drug abuse is a major risk factor for HIV-1 infection and opioids have been shown to alter the progression and severity of HAND. Buprenorphine is an opiate derivate used as a therapeutic for opioid dependency. It is a

partial agonist of MOR, and a full antagonist of KOR, opioid receptors, but its effects on monocyte migration relevant to the development of HAND have not been studied. We showed by FACS and qRT-PCR that human monocytes express MOR and KOR. We also demonstrated that treatment of human monocytes with buprenorphine and CCL2, a chemokine elevated in the CNS of people with HAND, reduces CCL2-induced migration. We showed that buprenorphine inhibits the phosphorylation of p38, necessary for monocyte migration, in part through the activation of MOR. The mature subpopulation of monocytes that expresses CD14 and CD16 are key mediators of neuroAIDS. We showed that these monocytes are increased in number in HIV infected people and even more so in HIV infected drug abusers, and that CD14+CD16+ monocytes from HIV-1 infected people have higher surface MOR and KOR. We will examine the effects of buprenorphine on the mechanisms of CCL2-mediated mature monocyte migration in the context of HIV-1 neuropathogenesis and determine the neuroprotective potential of this drug.

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DOPAMINE TRANSPORTER ACTIVITY IN THE STRIATUM OF HIV-1 TRANSGENIC FEMALE RATS

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HIV-associated proteins have been shown to interact with the sites of action of various drugs of abuse, including the dopamine transporter (DAT), and significantly reduced DAT function. However, the extent of DAT dysfunction in HIV-1+cocaine comorbidity is still unknown. The present experiments tested the transport of dopamine in rat brain slices from HIV-1 Tg females and control F344 rats during diestrous. We examined alterations in dopamine (DA) reuptake using Fast-scan cyclic voltammetry in control, HIV-1, cocaine-treated (5 µM) and HIV-1 + cocaine-treated striatal slices. In slices from control animals, cocaine treatment produced a persistent increase in extracellular dopamine levels, relative to untreated control slices (T80 for DA 2µM: 56.4 sec in cocaine-treated slice vs. 11.3 sec in untreated slice; about 5 times more). However, in HIV-1 Tg slices, the clearance parameters of DA were not affected by exposure of the

slice to cocaine (T80 for DA 2μM; 23.3 sec in cocaine-treated slice vs. 17.5 sec in untreated slice; about 1.3 times more); moreover, the DA signal in HIV-1 Tg was much less than that in the normal striatum, further confirming the dysfunctional role of the DAT in HIV-1 Tg animals. Analysis of DiOlistically labelled MSN dendrtic spines from these cocaine-treated slices further confirmed alterations in DA striatal reward systems. Taken together, the current study provides more evidence for the dysfunctional role of DAT in mediating DA reuptake within the striatum of HIV-1 Tg rats following exposure to the psychostimulant drugs such as cocaine.

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Sustained Release NanoART Formulation for the Treatment of NeuroAIDS

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A novel approach was developed for the co-encapsulation of AntiHIV drug (Tenofovir) and latency-breaking agent (Vorinostat) using magnetically guided Layer-by-Layer (LbL) assembled nanocarriers for the treatment of NeuroAIDS. Ultra-small iron oxide (Fe3O4) nanoparticles (10 nm) were synthesized and characterized. Layer-by-layer (LbL) technique was used to achieve sustained release profile, application of 2 bilayers {(Tenofovir +Dextran sulphate)2+Vorinostat} to magnetic nanoparticles (MNPs) resulted in 2.8 times increase in drug (Tenofovir) loading and also resulted in increasing the drug release period by 30 fold with 100% drug release in sustained manner over a period of 5 days with simultaneous stimulation of latent HIV expression. Nanoformulation showed good blood brain barrier transmigration ability (37.95±1.5%) with good in-vitro antiviral efficacy (~33% reduction of p24 level) over a period of 5 days after HIV infection in primary human astrocyte with good cell viability (>90%). In conclusion, the current work is an in-vitro proof-of-study demonstrating that latency breaking agents can be packaged into nanoparticles in conjunction with an antiretroviral drug (ARV). LbL arrangements of drug on MNP provides sustained release and; therefore, may improve patient’s adherence to therapy and may lead to better compliance. In future, in vivo studies will be performed using HIV infected SCID mice model to assess the free drug

amount (microdialysis) present in brain parenchyma to evaluate the therapeutic efficacy of the nanoformulation.

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DREADD activation of hippocampal astrocytes influences contextual fear conditioning

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Neuroinflammation is important in learning and memory processes. For example, our laboratory reported that severe stress induces a time dependent increase in interleukin-1β and that a central infusion of interleukin-1 receptor antagonist is sufficient to prevent the development of stress-enhanced fear learning (SEFL). Here, we tested whether activating hippocampal astrocytes altered the acquisition of contextual fear conditioning by employing Gq-coupled Designer Receptors Exclusively Activated by Designer Drugs (DREADDs) under an astrocyte promotor. All animals were infused with the viral vector, rAAV5/GFAP-HA-hm3D-IRES-mCitrine. Injectors were directed bilaterally at the dorsal hippocampus. In experiment 1, rats were injected with Clozapine-N-Oxide (CNO) or saline vehicle 2 hours prior to foot shock fear conditioning. In experiment 2, rats were injected with CNO or saline vehicle 6, 4, and 2 hours prior to foot shock fear conditioning. Twenty-four hours later, animals were tested for freezing behavior in the conditioning context, a measure of learned fear. In experiment 1, 2 hours of astrocyte activation did not alter fear learning. In experiment 2, our preliminary data suggests that 6 hours of astrocyte activation enhanced fear learning. We observed a significant interaction effect, $p = 0.019$, such that animals that received CNO followed by foot shock exhibited more freezing than animals that received vehicle followed by foot shock. These data suggest that astrocyte activity within the dorsal hippocampus is sufficient to induce a hypersensitivity to fear conditioning.

Mechanisms of antiretroviral drug-induced changes in amyloid precursor protein processing: Implications for HAND

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HIV-associated neurocognitive disorders (HAND) persist in 30-50% of HIV positive patients despite viral control by antiretroviral therapy (ART). Several studies indicate a potential role for antiretrovirals in persistence of HAND it evaolution from a subacute, subcortical dementia to a cortical, neurodegenerative disease. Based on their ability to induce ER stress in a wide variety of cell types, we hypothesized that HIV protease inhibitors (PI) induce ER stress in the CNS, resulting in chronic dysregulation of the unfolded protein response (UPR) subsequently altering amyloid precursor protein (APP) processing by inducing the β-site APP cleaving enzyme-1 (BACE1). Utilizing in vitro and in vivo models, we have shown that PIs induce neuronal ER stress leading to PKR-like ER kinase (PERK)-dependent phosphorylation of the eukaryotic translation initiation factor 2α(eIF2α), and enhanced translation BACE1. Additionally, we demonstrate enhanced Aβ production, by the PI, ritonavir, in primary rodent neuroglial cultures and Chinese Hamster Ovary (CHO) cells expressing human APP. Genetic excition of PERK in primary neurons abrogated the ability of PIs to induce the UPR, phosphorylation of eIF2α and translational upregulation of BACE1. Consistent with these findings, ARVs

administered to SIV-infected macaques resulted in elevated levels of BACE1 in the CNS coinciding with markers of neuronal damage. Altogether, these findings implicate PIs as potential mediators of neurodegeneration in HAND.

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HIV-1 Tat and gp120 Regulate Astrocyte Tissue Inhibitor of Matrix Metalloproteinases-1 (TIMP-1)

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While anti-retroviral therapy (ART) has improved the quality of life and survival of HIV-1 infected patients, HIV-1 associated neurocognitive disorders (HAND) remain a major problem in over 30% of cases. All forms of HAND are associated with CNS inflammation. Astrocytes are involved in signaling, homeostasis, and repair during CNS pathology; and some astrocytes become non-productively infected by HIV-1. The balance between matrix metalloproteinases (MMP) and their inhibitors must be tightly regulated during CNS inflammation. Tissue inhibitor of MMPs-1 (TIMP-1) protects human neurons from HIV-1-induced apoptosis and is mainly produced by astrocytes. Further, astrocyte TIMP-1 is differentially regulated during acute and chronic IL-1β-activation. However, the direct or indirect effects of astrocyte HIV-1 protein expression on TIMP-1 regulation are not well studied. To delineate downstream effects of HIV-1 Tat and gp120 on TIMP-1/MMP balance, primary human astrocytes were transfected with HIV-1 protein expressing plasmids. First, Tat and gp120 expression was compared using RT2-PCR and western blot. Cell viability and proliferation were evaluated as measures of cytotoxicity. Concurrently, time-dependent changes in TIMP-1 and CCL2 mRNA and protein levels were measured as indicators of astrocyte activation. Since C/EBPβ is a critical TIMP-1 regulator, alterations in C/EBPβ mRNA and protein levels were analyzed. We propose that TIMP-1 is regulated via HIV-1 protein expression in astrocytes that mimic viral CNS reservoirs, and may have implications in HAND neuropathogenesis.

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HIV-Tat alters Tau exon 10 splicing through dysregulation of SC35

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HIV-1 Tat is implicated in the neuronal degeneration process that affects more than half of HIV+ people. SC35, a splicing factor, localizes to nuclear speckles and plays key roles in Tau exon 10 alternative splicing. Alternative splicing of Tau exon 10 results in two Tau isoforms, 3R (exon 10 excluded) and 4R (exon 10 included), which possess three or four tubulin binding repeats, respectively. SC35-mediated splicing contributes to maintain an equal ratio of both these isoforms in the adult brain. Indeed, Tau exon 10 splice site silent mutations that alter this ratio contribute to frontotemporal dementia, Pick's Disease, and corticobasal degeneration. Here, we found that the Tau 3R/4R ratio is altered in brain tissues from individuals with HIV-encephalopathy, in Tat-transgenic mice, and in Tat-treated cell cultures. Altered Tau 3R and 4R ratio is accompanied with an aberrant increase in phospho-SC35 and SC35 disorganization in cellular cultures and in human brain samples. In addition, in vitro experiments show the negative effect of Tat on the SC35-mediated splicing of Tau exon 10. Tat-induced changes in SC35 homeostasis is likely mediated by alteration of two important kinases, GSK-3β and Drk1A. Altogether, our results demonstrate the ability of Tat to interfere with the cellular splicing machinery in uninfected cells. Since SC35 is also a component of the P-TEFb/RNA pol II complex and participates in the transcription in a manner similar to Tat on the LTR, we suspect a competition between Tat and SC35 for the same RNA structure and/or the same binding to the complex.

CCR5 antagonists preserved blood cells and diminished blood and CNS viremia in vivo

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The new class of antiretroviral drugs called entry inhibitors act by binding the CD4 receptor, CCR5 or CXCR4 co-receptors, to prevent viral binding and entry into cells. One of those entry inhibitors, maraviroc, is a small molecule CCR5 antagonist that is currently FDA-approved for the treatment of patients infected with macrophage (M)-tropic HIV strains. Because most HIV strains that cross the blood-brain barrier (BBB), enter the brain, and infect central nervous system (CNS) cells are M-tropic and use CCR5 to enter and infect target cells, we investigated the effects of maraviroc on HIV-1 infection in the blood and brain tissues in vivo, using an HIV/AIDS mice model: NOD/SCID gamma-/- (NSG) mice engrafted with human peripheral blood lymphocytes (PBL), and infected with HIV-1. Our study confirmed the antiviral activity of maraviroc, and showed that maraviroc inhibits HIV-1 infection of human macrophages in vitro. We further confirmed maraviroc antiviral activity in vivo, and showed that maraviroc treatment of infected animals prevented HIV-1-induced destruction of human CD4+ cells, human CD45+ cells, and human CD195+ cells in these animals. Quantification of HIV-1 gag and p24 antigen levels showed that maraviroc significantly decreased viremia in the blood, spleen, and brain tissues of infected animals. These data suggest that CCR5 antagonists could be protective against HIV-1 infection of the CNS.

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CCR5 antagonists preserve the brain vascular endothelium in HIV/AIDS animal models

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HIV-1 induce injury and dysfunction of the blood-brain barrier (BBB) in the early stages of infection, and this result in the entry of virus and infected cells into the CNS, infection of resident brain macrophages and microglia, and HIV-associated neurocognitive disorders (HAND). Our previous works demonstrated that STAT1 and Rac1 signaling

modulate BBB dysfunction following HIV-1 infection and endothelial-monocytes / macrophages interactions, and we further demonstrated that both CCR5 neutralizing antibodies and CCR5 antagonists significantly diminished HIV-1-induced BBB dysfunction. In the present study, we tested this hypothesis in vivo using an HIV/AIDS mice model: NOD/SCID gamma-/- (NSG) mice engrafted with human peripheral blood lymphocytes (PBL), and infected with HIV-1. Treatment of infected animals with the CCR5 antagonist maraviroc preserved the brain vascular endothelium, and prevented HIV-1-induced downregulation of the tight junction proteins claudin-5, ZO-1, and ZO-2 on the animals' brain vascular endothelium. These data suggest that the CCR5 antagonist maraviroc can preserve the brain endothelium in vivo, which could diminish viral entry into the CNS and results in protective effects against HAND.

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Exosomes from HIV-1 Infected Cells Stimulate Production of Pro-inflammatory Cytokines through TAR RNA

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HIV-1 infection results in a chronic illness since long-term HAART can lower viral titers to an undetectable level. However, discontinuation of therapy rapidly increases virus burden. We have previously shown that exosomes containing viral RNA enhance susceptibility of undifferentiated naive cells to HIV-1 infection. Exosomes derived from virally infected cells have been shown to contain viral non-coding RNAs as well as microRNAs (viral or cellular). The most abundant HIV-1-derived non-coding RNA and miRNA, first reported by us and later by others using deep sequencing, is the TAR RNA. TAR RNA influences both viral and host cell transcription and influences apoptosis in the context of cellular stress. The current study will discuss how exosomes from HIV-1 infected primary cells are highly abundant with TAR RNA as detected by RT-real-time PCR. Interestingly, up to a million copies of TAR RNA per microliter were

also detected from HIV-1 infected humanized mice suggesting that HIV-1 TAR RNA may be stable in vivo. Incubation of exosomes with primary macrophages resulted in a dramatic increase of proinflammatory cytokines, IL-6 and TNF-α indicating that exosomes containing TAR RNA could play a direct role in control of cytokine gene expression. The intact TAR molecule was able to bind to PKR and TLR3 effectively, whereas 5' and 3' stems bound best to TLR3, 7, 8 and none to PKR. Binding of TAR to PKR did not result in its phosphorylation and therefore TAR may be a dominant negative decoy molecule. The single stranded 5' or 3' stem RNA binding to TLRs activates the NF-κB pathway and regulate cytokine expression. Collectively, our results imply that exosomes containing TAR RNA could directly affect the proinflammatory cytokine gene expression and may explain a possible mechanism of inflammation observed in HIV-1 infected patients.

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Use of membrane proteome to find new and novel targets expressing on the surface of HIV-1 infected cells.

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Many cellular cofactors have been documented to be critical for various stages of viral replication. Using high throughput proteomic assays, we have previously identified Bruton's tyrosine kinase (BTK) as a host protein that was uniquely up-regulated in the plasma membrane of HIV-1 infected T-cells. Here, we have further characterized the BTK expression and show that this cellular factor is specifically expressed in infected myeloid cells. Significant up-regulation of the phosphorylated form of BTK was observed in infected cells. Using size exclusion chromatography, we found BTK to be virtually absent in the uninfected U937 cells, however new BTK protein complexes were identified and distributed in both high molecular weight (~600 kDa) and a small molecular weight complex (~60-120 kDa) in the infected cells. BTK levels were highest in cells either chronically expressing virus or induced/infected myeloid cells and that BTK translocated to the membrane following induction of the infected cells. BTK

knockdown in HIV-1 infected cells using siRNA resulted in selective death of infected, but not uninfected, cells. Using BTK specific antibody and small molecule inhibitors including LFM-A13 and a FDA approved compound, Ibrutinib (PCI -32765), we have found that HIV-1 infected cells are sensitive to apoptotic cell death and result in a decrease in virus production. Overall, our data suggests that HIV-1 infected cells contain unique membrane proteins that can be targeted and they are sensitive to treatments against these targets (i.e., BTK) when expressed in infected cells.

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In vitro & In-vivo Cytotoxic Evaluation of Magneto-Electric Nanoparticles for the CNS Delivery

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Our laboratory has explored potentials of magneto-electric nanoparticles (MENPs) as in-vitro on-demand controlled release of drugs for the treatment of neuroAIDS and other CNS diseases. Aim of this research was to explore cytotoxicity of MENP nanoformulation both in vitro & in vivo. In this work, we have explored the dose dependent (50-250 µg/mL) in vitro and in vivo cytotoxicity (5-20 mg/kg) of MENPs of BaTiO3 CoF2O4 in primary brain cells (neurons & astrocytes) and Balb/C mice to assess general and organ specific cytotoxicity respectively. Biocompatibility results showed that treatments with different concentrations of MENP nanoformulation do not show any sign of cytotoxicity and the percentage of viable cells (> 90%) was similar to untreated control. Further, pilot in vivo studies with Balb/C mice were performed for MENP dose optimization, a dose of 10mg/kg showed the best result. Further, organ specific (brain, kidney, liver and spleen) and peripheral blood parameters (Hepatic and renal function test) toxicity were tested using standard H&E staining method and blood profiling method respectively. In conclusion, we can say that results suggest that doses of MENPs nanoformulation are nontoxic and have no significant safety concerns. Thus,

suggesting potential application of MENPs for site-specific on-demand controlled delivery of therapeutics across BBB for the prevention CNS diseases.

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Acute phase protein lipocalin 2 and CCR5 control microglial activation and neuronal injury in an in vivo model of HIV-induced brain injury.

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Increased numbers of activated microglia are a hallmark of HIV-1 associated brain injury. Both, CXCR4- and CCR5-utilizing viruses and their envelope protein gp120 trigger neurotoxin production in microglia and macrophages. Here we show in a transgenic model of HIV-associated brain injury that the genetic knockout of CCR5 abrogates microglial activation and neuronal damage induced by a CXCR4-utilizing viral envelope gp120. A genome-wide CNS gene expression analysis reveals that brains of CCR5 wild-type (WT) and CCR5KO gp120tg mice express markers of an innate immune response. One of the most significantly up-regulated factors is the acute phase protein lipocalin-2 (LCN2). Using cerebrocortical cell cultures containing neurons, astrocytes and microglia, we find that LCN2 exerts neurotoxicity in a CCR5-dependent fashion whereas inhibition of CCR5 itself does not affect neurotoxicity of the CXCR4-utilizing gp120. However, disruption of CCR5 signaling during combined exposure to LCN2 and a CXCR4-utilizing gp120 causes a loss of microglia and protects neurons from toxicity, thus recapitulating the finding in CCR5-deficient gp120tg mouse brain. In summary, our study provides evidence for an indirect pathological role of CCR5 in HIV-associated brain injury and an unexpected LCN2-dependent, protective mechanism of CCR5 inhibition.

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HIV-Tat 101 protein regulation of electrophysiological properties of midbrain dopaminergic neurons

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HIV-1 transactivator of transcription (HIV-1 Tat) protein has been shown to play a critical role in perturbations of the dopamine (DA) neurotransmission in the brain. The dopamine (DA) transporter (DAT) is a major regulator of extracellular dopamine and thus dopamine neurotransmission in the brain and it is implicated in neurological and neuropsychiatric disorders such as Parkinson's disease and drug addiction. Methamphetamine (METH) is a DAT target and a prevalent drug of abuse amongst HIV population. Recent reports suggest HIV-1 Tat protein enhances METH-induced impairment of dopaminergic transmission, albeit with less understood mechanism. It is believed that HIV-1Tat protein influences [3H]DA uptake, with unknown mechanism. There is no information available regarding the specific effect of HIV-1Tat on METH-regulation of DAT activity. Our preliminary data suggest METH increases the DAT-mediated inward current, increases the spontaneous firing activity of dopaminergic neurons and increases DAT-mediated DA efflux. The major goal of this study is to determine whether and how HIV-1Tat protein influences basal DAT activity or METH-regulation of DAT activity. We used patch-clamp electrophysiology in whole cell configuration and live-cell confocal microscopy to address this critical knowledge gap. Our preliminary data suggests that intracellular HIV-1 Tat protein (200 ng/ml) delivered directly into the dopamine neurons via the patch electrode attenuates METH (10μM) induced, DAT-dependent DA efflux from midbrain dopamine neurons.

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β -Amyloid, HIV-1 Infection and Drugs of Abuse Induced Neurodegeneration and Protective Effects of Ashwagandha

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Alzheimer's disease (AD) is characterized by abnormal accumulation of extracellular amyloid plaques and intracellular neurofibrillary tangles throughout cortical and limbic brain regions. Ashwagandha (ASH) is used widely in Ayurvedic medicine as a nerve tonic and memory enhancer. However, there is paucity of data on potential neuroprotective effects of ASH against β' Amyloid (Aβ) induced neuropathogenesis. In the present study, we have tested the neuroprotective effects of ASH extracts and its constituent Withanolide A (WA) against Aβ induced toxicity, HIV-1Ba-L (clade B) infection and the effects of drugs of abuse using a human neuronal SK-N-MC cell line. Aβ when tested individually, induced cytotoxic effects in SK-N-MC cells as shown by increased trypan blue stained cells. However, when ASH was added to Aβ treated cells the toxic effects were neutralized. This observation was supported by cellular localization of Aβ, MTT formazan exocytosis, and the levels of acetylcholinesterase activity, confirming the chemopreventive or protective effects of ASH against Aβ induced toxicity. Similar results were obtained in cells infected with HIV-1Ba-L (clade B) as well as in cells treated with Cocaine (COC) and Methamphetamine (METH). Also, WA, a purified constituent of ASH, showed same pattern using MTT assay as a parameter. These results suggests that neuroprotective properties of ASH observed in the present study may provide some explanation for the ethnopharmacological uses of ASH in traditional medicine for cognitive and other HIV associated neurodegenerative disorders.

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Comparative analysis of lymphocyte and cytokine profiles in peripheral and enteric lymph nodes of SIV-infected Chinese rhesus macaques

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Chronic immune activation during HIV infection is associated with peripheral CD4+ T cell depletion and disease progression. However, it remains to be determined whether immune activation contributes to CD4+ T cell loss in secondary lymphoid tissues, particularly in gastrointestinal (GI) system. Thus, we examined the expression of CD4+ T cells in the lymph nodes (LNs) and intraepithelial lymphocytes (IELs) in the GI system of SIV-infected macaques. We also measured the expression of immune activation markers (CD38, CD69 and HLA-DR) on CD4+ and CD8+ T cells in these tissues. We observed that the lymphoid tissues (colon, mesenteric and iliac LNs) from the GI system of SIV-infected macaques had significantly lower expression of CD4+ T cells than those (axillary and inguinal LNs) from the peripheral tissues. The severity of CD4+ T cell loss was negatively associated with the levels of the activation markers in GI LNs. In addition, the median SIV loads in the mesenteric LNs were higher than peripheral LNs, which was positively associated with the levels of inflammatory cytokines (TNF-α, IL-6 and IL-8). These observations indicated that the immune activation of GI LNs contributes to CD4+ T cell depletion and viral replication in lymphoid tissues.

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Combinatorial peripheral and brain humanization in immunodeficient mice

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Despite the availability of humanized mice as models for human immunodeficiency virus (HIV) infection; limitations abound for mimicking cell brain migration and virus-induced inflammation. We now posit that the best means to reflect HIV-1 associated encephalopathy the brain and the immune system must be reconstituted with primary human cells. This is based on region and graft acquisitions for the brain and the

acquisition of human progenitor cells to adequately repopulate the lymphoid system. To address such limitations we sought to simultaneously humanize the peripheral human system and brain. Human astrocyte progenitor cells (APC) were isolated then cultivated from human fetal tissue provided by the University of Washington Medical Center, Seattle. APC were engrafted into neonatal NSG mice simultaneously transplanted with CD34+ hematopoietic stem cells (HSC) isolated from human fetal liver from the identical donor. Following cell engraftments at eight weeks demonstrated by successful human hematopoiesis blood was obtained then evaluated by flow cytometry. Brain tissue was examined showing large number human glia identified as astrocytes, which were well accepted without evident mouse microglial activation. Moreover, human hematopoiesis was observed in these mice with a considerable number of human T and B cells and monocytes. Thus, reconstitution of mouse with human glial cells and peripheral hematopoiesis offers a unique animal model to systematically explore the pathological mechanism underlying human-specific viral infections, including HIV, JC and cytomegalovirus.

Prolonged intrathecal exposure to gp120 induces HIV-related neuropathic pain and spinal pharmacological characteristics

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Neuropathic pain in patients with HIV/AIDS may be initiated by chronic HIV glycoprotein gp120 exposure. To further model the predominant clinical scenario of chronic HIV-related pain, we investigated the effects of prolonged exposure to HIV gp120 on the acute-to-chronic pain transition, the neurochemical changes and spinal pharmacological characteristics in rats. Repeated administration of intrathecal recombinant HIV gp120, but not single injection induced a persistent mechanical allodynia, beginning on day 5 following gp120 compared to vehicle group. The lowest threshold was observed at day 10 even if the delivery of gp120 was stopped at day 5. HIV gp120 increased the expression of spinal glial marker proteins and TNFα. The chronic gp120 administration induced the loss of intraepidermal nerve fiber (IENF) density in the hindpaw, suggesting peripheral neuropathy developed. Pharmacological studies showed that spinal TNFα,

CXCR4, and Wnt systems were involved in the neuropathic pain state. The present studies demonstrated that chronic spinal exposure to HIV gp120 induce prolonged mechanical allodynia accompanied by spinal and peripheral inflammatory neuropathology, which resembles several important aspects of HIV-1-related sensory neuropathy in people and provides new insights on mechanism-based therapies designed to prevent/treat neurological deterioration associated with HIV infection in people.

Activity of mitochondrial fission protein is involved in HIV gp120-induced neuropathic pain in rats through spinal mitochondrial superoxide

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BACKGROUND: Mitochondria are dynamic organelles, and their fusion and fission regulate cellular signaling, development, and mitochondrial homeostasis. The most common complaint of human immunodeficiency virus-sensory neuropathy (HIV-SN) is pain on the soles in HIV patients, but the exact molecular mechanisms of painful HIV-SN are not clear. In the present studies, we investigated the role of mitochondrial dynamin-related protein 1 (Drp1, a GTPase that mediates mitochondrial fission) in the HIV coat glycoprotein gp120-induced neuropathic pain state. RESULTS: Neuropathic pain was induced by the application of recombinant HIV-1 envelope protein gp120 into the sciatic nerve. Mechanical threshold was tested using von Frey filaments. Intrathecal administration of either antisense oligodeoxynucleotide (ODN) against Drp1 or mitochondrial division inhibitor-1 (mdivi-1) decreased mechanical allodynia in the gp120 model. Intrathecal Drp1 antisense ODN decreased the spinal expression of increased Drp1-immunoreactivity induced by peripheral gp120 application. Moreover, both intrathecal Drp1 antisense ODN and mdivi-1 reversed the upregulation of mitochondrial superoxide in the spinal dorsal horn in the gp120 neuropathic pain state. CONCLUSIONS: These data suggested a substantial role of mitochondrial division in the HIV gp120-related neuropathic pain state, and provide evidence for a novel approach to treating chronic pain due to HIV-SN.

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ROLE OF ER-STRESS AND NEUROINFLAMMATION IN HUMAN ASTROCYTES TREATED WITH HIV-1 TAT AND GP120

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It is suggested that the degree of Neuro-AIDS vary according to HIV-1 clades. The clades of HIV-1 B and C account for the majority of HIV-1 infections, clade B being the most immuno-neuropathogenic. There are no established mechanisms of HIV-mediated neuropathogenesis, and therefore, it remains the subject of active research. Based on that, we hypothesize that HIV-1 Tat B and Gp120 clade B induce a pro-apoptotic and a pro-inflammatory response in human astrocytes vs. HIV-1 clade C proteins. We used a proteomic approach on human astrocytes treated with Tat and Gp120 proteins from HIV-1 clades B and C separated by two-dimensional gel electrophoresis followed by liquid chromatography-tandem mass spectrometry for protein differentiation. Cytokine and chemokines analysis were performed using cytometric bead assay. Among the significantly upregulated proteins by HIV-1 Tat and Gp120 clade B are pro-apoptotic, endoplasmic reticulum and oxidative stress markers and numerous metabolic factors. Moreover, HIV-1 clades B proteins induced a pro-inflammatory response with the expression of key inflammatory cytokines including IL-1β, IL-6 and IL-8. These data suggest that HIV-1 Tat B/C and Gp120 B/C induce a differential pro-inflammatory response and pro-apoptotic protein profile in human astrocytes. Our findings demonstrate that HIV-1 clade B induce an inflammatory, ER and oxidative stress mediated pro- apoptotic response while HIV-1 clade C seems to be associated with anti-apoptotic mechanisms in human astrocytes.

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Morphine potentiates LPS-induced autophagy initiation but inhibits autophagosomal maturation in murine macrophages through TLR4 dependent and independent signaling

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Morphine produce powerful analgesia that is effective in treating various types of pain. Our previous studies show that morphine impairs host innate immune response and increases susceptibility to opportunistic infection. Recently, autophagy has been shown to be an innate defense mechanism against bacterial infection. However, the mechanism by which morphine treatment modulates LPS induced autophagy leading to autophagolysosomal induced bacterial killing is not known. Macrophages were transfected with GFP-mcherry-LC3 simultaneously to monitor autolysosome formation and subsequent degradation events. Morphine potentiates LPS-induced autophagyinitiation, however, morphine inhibited LPS-induced autophagosome maturation and autophagolysosomal fusion as indicated by the failure to recruit LAMP1 into autophagosome and reduced degradation of SQSTM1/p62 protein level. Morphine modulation of LPS-induced autophagosome maturation visualized using co-localization of GFP-mcherry-LC3 is TLR4 independent but mediated through μ opioid receptor signaling. Morphine and LPS combination treatment significantly increased Streptococcus pneumoniae load when compared with LPS treatment. These observations imply that although morphine treatment facilitates LPS-induced autophagy, it inhibits autophagolysosomal fusion leading to decreased bacterial clearance and increased bacterial load. This study gives us new insight into the possible mechanism(s) that the observed increased in the susceptibility to infection and the prevalence of persistent infection in the drug abusing population.

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Soybean-derived Bowman-Birk Inhibitor (BBI) Inhibits HIV infection of Human Macrophages

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The Bowman-Birk inhibitor (BBI) is a soybean-derived protease inhibitor that has anti-inflammatory effect in both in vitro and in vivo systems. Inflammation and immune activation are believed to be driving power for HIV disease progression. Thus, we investigated effect of BBI on HIV infection of peripheral blood monocyte-derived macrophages. We demonstrated that BBI could inhibit HIV infection of macrophages. Investigation of mechanism(s) of BBI action on HIV showed that BBI induced the expression of IL-27, a newly identified anti-HIV cytokine, and the production of IFN inducible antiviral factors, including APOBEC3G (A3G), myxovirus resistance protein 2 (Mx2), 2', 5'-oligoadenylate synthetase (OAS-1), Virus inhibitory protein (viperin), protein kinase R (PKR), IFN-stimulated gene 15 (ISG15) and the IFN-stimulated gene 56 (ISG56). In addition, BBI-treated macrophages expressed increased levels of CC chemokines (RANTES, MIP-1α, MIP-1β), the ligands for HIV entry co-receptor CCR5. BBI also induced the expression of tetherin, intracellular factor that restricts HIV release from infected cells. The finding that BBI could inhibit HIV infection of macrophages at different stages of viral infection, suggests that BBI may have therapeutic potential in the treatment of HIV disease.

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Co-morbid HIV-Tat and morphine attenuate human neural stem cell neurogenesis and alter proteins regulating neural functions

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Prevalence of neuro-developmental disorders in perinatally HIV-infected children or in infants born to opioid abusing mothers suggest perturbations in neural stem/progenitor cell (NPC) functions further leading to neurobehavioral abnormalities. Since co-morbid HIV and opioids have been shown to affect the proliferative potential of NPCs, we investigated if multipotency of these cells is compromised. Human fetal NPCs were exposed to co-morbid HIV-protein, Tat and morphine and differentiation into neuronal

lineage was assessed. Comprehensive gene analysis revealed reduced expression of genes involved in maintenance of NPC pool and initiation of differentiation, and simultaneous increase in certain basic helix-loop-helix (bHLH) transcriptional repressors such as Hey and Hes. Further programming of NPCs into neuronal lineage in presence of co-morbid HIV-Tat and morphine exposure revealed compromised neurogenesis which may serve as a confounding factor for HIV Associated Neurocognitive Disorders (HANDs). Following neurogenesis for up to two weeks in culture with simultaneous HIV-1 Tat and morphine exposure revealed down-regulation of several genes involved in cell adhesion, establishment and maintenance of neuronal connections and synapse assembly. Ours is the first study which has looked into neuronal differentiation of human NPCs with co-morbid HIV-1 Tat and morphine exposure and provides a new facet to HIV-drug abuse co-morbidity that may have far reaching clinical consequences both in paediatric as well as adult neuroAIDS.

TGF-betaR1 blocker decreases inflammatory cytokines expression in neurons exposed to astrocytes expressing Nef

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Milder forms of HIV-1 associated neurological disorders (HAND) represent one of the prevalent comorbidities that patients undergo in the current cART era. The viral protein Nef, a neurotoxin that causes learning deficiencies, is produced in the brain even when the virus is not detected. Our laboratory has published data from experiments done in an animal model of Nef neurotoxicity suggesting that TGF-beta is associated with learning impairments and correlates with increased CCL2 expression. In order to study the effect of Nef on the TGF-beta signaling pathway and inflammation, we used a co-culture system and a TGF-betaR1 blocker (SD208). After primary neurons were grown to confluence, primary astrocytes were transfected with either Nef or GFP and placed on culture inserts in each well. RNA was isolated and RT-PCR was used to measure gene expression of TGF-beta, TGF-betaR1, CCL2, IL6, and TNF at 24 and 48 hours. Significant results were observed at 48 hours where inflammatory cytokines decreased

in the presence of the blocker. These data indicate that expression of Nef in astrocytes alone is capable of activating the TGF-beta gene while inducing inflammation in the brain and that the signaling pathway is involved in Nef neurotoxicity. Further experiments will include analysis of cytokine protein levels in the presence of the blocker. Understanding how Nef alters the TGF-beta signaling pathway may be critical for the development of treatments that can target areas in the brain were cART has not been able to successfully exert an effect.

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Tight junction protein expression in a blood-brain barrier model upon exposure to HIV-1 Tat and/or morphine

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Injection drug use has been directly linked to the acquisition of approximately one-third of human immunodeficiency virus type 1 (HIV-1) cases resulting in acquired immunodeficiency syndrome (AIDS) in the United States. To make matters worse, opioid abuse within this population exacerbates disease progression, including increased viral replication and peripheral viral load, as well as incidence and severity of neurocognitive impairment, as compared to non-users. Breaching of the 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 various drugs of abuse, have been implicated in the observed compromise of the BBB. Previous studies suggest 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 chronic Tat and/or morphine exposure on tight junction protein (TJP) expression of the BBB. Changes in mRNA transcripts of TJPs were observed throughout the course of exposure. At the protein level, TJP expression and localization was analyzed following cell fractionation and western immunoblot analysis. Overall, these studies demonstrate that exposure to Tat and/or morphine induces changes in TJP expression patterns at both the mRNA and protein level.

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CNS Exposure to HIV-1 Tat Protein Modulates Forebrain Glutamate Levels and Increases Depression-like Behavior

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We hypothesized that HIV-1 Tat expression in brain would modulate glutamate levels and promote behavioral depression. 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 depression-like behavior in the tail-suspension test (TST), saccharin consumption test and forced swimming test (FST). Furthermore, magnetic resonance spectroscopy (MRS) was used to determine whether Tat protein alters medial frontal cortex intracellular levels of glutamate and N-acetylaspartate (NAA). In behavioral testing, GT-tg bigenic mice expressing Tat protein for 7 d demonstrated significant increases in time spent immobile during the TST and a significant decrease in saccharin consumption as compared to saline-treated littermates lacking Tat, both characteristic of increased depression. MRS studies of medial frontal cortex found trend and significant decreases in glutamate and NAA, respectively, in GT-tg mice treated 7d with Dox versus baseline measures in these mice. Overall, these data suggest that exposure to HIV-1 Tat protein in mouse brain is sufficient to increase behavioral depression. Moreover, the Tat-induced decrease in MRS metabolites

suggests biological mechanisms by which HIV infection may increase the vulnerability to depression, which could be exploited as via specific therapeutic interventions. Pilot data examining the effects of treatment with the NMDA-receptor antagonist ifenprodil on Tat-induced effects will be discussed.

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Plant-based product Apigenin attenuates EAE severity through the modulation of immune cell function.

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The use of Apigenin, a naturally occurring plant flavone, for centuries to treat asthma, Parkinson's disease, neuralgia, and shingles, indicates its importance in the regulation of inflammation. However, its effect on dendritic cells (DC) that maintain the critical balance between an immunogenic and tolerogenic immune response especially in neuroinflammation is relatively unknown. To test if Apigenin treatment ameliorates disease after onset of experimental autoimmune encephalomyelitis (EAE), an animal model of multiple sclerosis, C57BL/6 mice immunized with MOG35-55 were treated with Apigenin. A significant reduction in severity of EAE progression was observed in the treated mice with disease peak lasting only a day. Splenocytes isolated from Apigenin treated EAE mice show decreased expression of α4 integrin and increased DCs and macrophages compared to untreated EAE mice. This correlated with immunohistochemistry findings of decreased immune cell infiltration and reduced demyelination in the CNS. In vitro, Apigenin inhibited TNF-α and IL-6 secretion in mice splenic DCs stimulated with LPS and the cell surface expression of MHC II and CD86 molecules on bone marrow derived DCs. These results indicate a protective role of Apigenin against the neurodegenerative effects resulting from the entry of DC stimulated pathogenic T cells into the CNS. Apigenin can thus serve as a potential

therapy for neuroinflammatory disease through its regulation of immunogenic T cell response.

Opioid Exacerbation of Gram-positive sepsis, induced by Gut Microbial Modulation, is Rescued by IL-17A Neutralization

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Sepsis is the predominant cause of mortality in the ICUs and opioids are the preferred analgesic in this setting. In this study, we first demonstrate that morphine alone altered the gut microbiome composition and selectively induced gram-positive sepsis in mice. Using a murine model of poly-microbial sepsis, we further demonstrate that morphine treatment led to predominantly gram-positive bacterial dissemination. Dissemination of gram-positive bacteria in morphine-treated animals led to activation of TLR-2 and induced sustained up-regulation of IL-17A and IL-6. In contrast, in placebo-treated animals the immune activation induced only transient IL-6 expression. We subsequently show that overexpression of IL-17A compromises intestinal epithelial barrier function, sustained bacterial dissemination and elevated systemic inflammation. Neutralization of IL -17A protected barrier integrity and improved survival in morphine-treated animals. Ex vivo studies using TLR-2 knockout and wild type cells demonstrate that TLR-2 expressed on both dendritic cells and T cells play essential roles in IL-17A production in the mesenteric lymph node. In addition, we show that intestinal sections from patients on chronic opioids and sepsis exhibit similar disruption in gut epithelial integrity, establishing the clinical relevance of this study. This is the first study to provide a mechanistic insight into opioid exacerbation of sepsis and show that neutralization of IL-17A might be an effective therapeutic strategy to manage gram-positive sepsis in patients on opioid regimen.

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Brain Injury Induces Neuroinflammation in the Nucleus Accumbens that Coincides with Enhanced Addiction-Like Behavior in Adolescent Mice

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Clinical psychiatric disorders of depression, anxiety, and substance abuse are most prevalent following traumatic brain injury (TBI). Preclinical research has largely focused on depression and anxiety post-injury; however, virtually no data exists examining whether the preference for illicit drugs of abuse is affected by traumatic injury to the developing adolescent brain. Using the controlled cortical impact model of TBI coupled with the conditioned place preference (CCP) assay, we test the hypothesis that brain injury during adolescence exacerbates the reinforcing properties of cocaine in adulthood by affecting function of the reward pathway. Six-week old, male C57BL/6 mice sustained a single impact TBI of varying severity (mild or moderate) to the right somatosensory cortex. CPP pre-testing began 2 weeks post-TBI, followed by 6 days of intraperitoneal cocaine administration (10 mg/kg). The place preference shift was significantly enhanced in all treatment groups receiving cocaine compared to saline controls; furthermore, a moderate TBI during adolescence caused a significant increase in the place preference shift compared to non-surgical cocaine controls. Few reports have examined the presence and potential of mesolimbic neuropathology following brain injury. Using GFAP and IBA-1 immunofluorescence, we have observed persistent neuroinflammatory responses in the nucleus accumbens following TBI. These results suggest that sustaining a moderate TBI during adolescence may augment addiction-like behavior in adulthood possibly related to mesolimbic neuroinflammation.

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Cigarette smoke condensate (CSC) differentially regulates PRDX6 and catalase expression in exosomes secreted from monocytes-derived macrophages

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Smoking is known to exacerbate HIV pathogenesis. However, its underlying mechanism is poorly known, except the fact that oxidative stress (OS), perhaps through cytochrome P450 (CYP), is associated with smoking-mediated HIV pathogenesis. Exosomes, the membrane bound nano-vesicles, produced by many cell types, play a crucial role in inter-cellular cell signaling and are believed to be involved in HIV pathogenesis. However, no data is available whether exosomes contribute to the disease progression in HIV+ smokers. In the present study, we studied the role of cigarette smoke condensate (CSC) on the secretion of U937 monocytic cells-derived exosomes and exosomal proteins. First, we showed that mRNA level of CYP1A1 is 5-fold higher, whereas mRNA of CYP2A6 and CYP2E1 are 20-fold higher in exosomes than U937 cells. Likewise, exosomal AOEs such as SOD1 and SOD2 exhibited 5-fold, whereas, catalase, GSTK1 and PRDX6 showed a dramatic 100-500-fold increase in mRNA in exosomes comparted to U937 cells. Furthermore, results from western blot showed that only catalase and PRDX6 are detectable in U937 cells-derived exosomes. Importantly, CSC treatment showed ~2 fold increase in catalase protein, but almost completely abolished PRDX6 protein in the exosomes without affecting their levels in U937 cells. Overall, these results suggest that exposure to CSC alters exosomal catalase and PRDX6. Current investigations are underway to assess the role of CSC-mediated secretion of exosomes and exosomal catalase and PRDX6, and their effects on HIV pathogenesis.

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Learning and Memory Impairment in HIV-1-transgenic (Tg)26 mice is Associated with Reduced Levels of Brain-Derived Neurotrophic Factor

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HIV-1 associated neurocognitive disorders (HAND) continue to be a major concern in the infected population, despite the widespread use of anti-retroviral therapy. Although majority of the evidence on HIV-1-neurotoxicity are based on the effects of Tat and gp120, neurotoxic effects of other viral peptides such as Nef and Vpr have also been reported. It is therefore important to understand how different viral factors of neuropathogenic potential act-in-concert to mediate their effects in the central nervous system, in order to develop an efficient therapy against HAND. Here we analyzed learning and memory in transgenic (Tg)26 mice that have a 7.4-kb pNL4-3 HIV-1 provirus lacking a 3.1-kb sequence encompassing parts of the gag-pol region. We provide the first evidence that 18-22 weeks old Tg26 males show an impaired spatial reference memory and contextual-fear memory, compared to their wild-type littermates. Immunohistochemical and stereological analyses showed similar number of neurons in the hippocampal CA1, CA3 and DG regions between all animal groups. However, Tg26 males showed elevated expression of pro-inflammatory genes (TNFα, IL-6 and COX-2) in the pre-frontal cortex, but not in the hippocampus. Moreover, real-time PCR analysis of hippocampal RNA isolated from Tg26 males indicated an increased expression of profilin-1 and reduced expression of BDNF genes, both of which are associated with shrinkage of neuronal spine density and impaired memory. Thus, therapeutic approaches aimed at restoring mature BDNF levels in the brain might be beneficial in the treatment of HAND.

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The gut microbiota as a therapeutic intervention for sustained attention deficits in the HIV-1 transgenic rat

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In the era of combination antiretroviral therapy, deficits in executive function develop more rapidly during the progression of HIV-1-associated neurocognitive disorders (HAND) than those of other cognitive domains. We 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 S-equol, a metabolite produced via the gut microbiome following ingestion of soy isoflavone daidzein, administered at 6-8 months of age. In another experiment, performance on the sustained attention task was assessed while the animals were administered S-equol (0.2 mg, oral pellet) or sucrose for 60 days, beginning at 2-3 months of age. Rats were trained to discriminate light signals (100, 500, or 1000 msec) from non-signals. 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, HIV-1 Tg animals that received S-equol exhibited a high level of accuracy across all signal durations, whereas control animals that received S-equol failed to reliably detect the 100 msec signal throughout the entire testing period. Thus, the 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. The microbiota-gut-brain axis may provide an important therapeutic approach for HAND.

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Morphine upregulates Ferritin Heavy Chain in primary inhibitory neurons in a μOR/g protein dependent manner

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Current HIV therapies have dramatically improved patients' quality of life, but HIV-associated neurocognitive disorder remains a prevalent and challenging problem to address in the clinic. HIV+ patients who abuse drugs, particularly opiates, can show

enhanced cognitive impairment compared to those who have not, suggesting an additive effect of these maladies. Morphine, via μOR, has been shown to upregulate Ferritin Heavy Chain (FHC) in neurons of multiple species, including humans. Although this subunit of Ferritin is classically associated with iron oxidation/sequestration, our studies have shown FHC interacts with the chemokine receptor CXCR4, resulting in a blockade of homeostatic signaling and a reduction in dendritic spine density, which correlates with cognitive decline. The studies herein show that FHC upregulation depends on ÂμOR associated G proteins and it is not a general effect. Indeed, primary astrocytes are not affected and the degree of change varies among cortical neurons. Imaging studies of single neurons show that FHC is expressed in the soma under basal conditions, while morphine causes expression to become apparent in processes, which is consistent with CXCR4 distribution in these cells. Western blot studies confirmed increased FHC levels in cytosolic protein extracts of neurons, and no changes in the nucleus. Finally, morphine-induced FHC increase is variable at the single cell level; neurons that highly express the GABA transporter GAT-1 preferentially upregulate FHC, suggesting inhibitory neurons may be a more susceptible cell population.

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Trafficking of conventional dendritic cells (DCs) into the central nervous system in response to SIV and Morphine creating viral reservoir in follicular DC within cervical lymph nodes of Rhesus Macaques

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Neuroinvasion by HIV leads to neurocognitive diseases and alters the permeability of the BBB. Conventional dendritic cells (cDCs) infiltrating the CNS can potentially

encounter HIV from infected perivascular macrophages lining the BBB. They can thereafter carry HIV to cervical lymph nodes (CxLNs), where HIV particles will be transmitted to CD4 T cells and eventually get trapped in CD35+ follicular DCs (fDCs) to create a viral reservoir within the germinal centers. HIV patients using drugs of abuse such as morphine can further compromise the BBB and enhance infectivity of the CNS. Research demonstrating the presence and role of DCs in the CNS and lymphoid organs during HIV infection has not well established. In this respect, we explored the presence of DCs in the brain parenchyma and CxLNs of rhesus macaques infected with SIV and administered with morphine. Cells positive for cDC markers were consistently found in the brain parenchyma of SIV-infected macaques and enhanced within infected macaques given morphine. Examination of CxLNs in SIV-infected macaques confirmed presence of SIV p27+ cDCs, and CD4+ T-cells entering CD20+ germinal centers further trapping of virus within CD35+ fDCs. Ongoing studies are assessing the effect of SIV chronic infection and Morphine on fDC viral entrapment. These results provide first evidence of DC trafficking and containment of SIV particles within fDCs of cervical lymph nodes in the context of NeuroAIDS vis-à-vis drugs of abuse.

Striking a balance: neural and behavioral implications of the interplay of stress and immunity in health and disease

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Although antiretroviral therapy (ART) has dramatically increased the life expectance of people living with HIV (PLWH), PLWH still suffer from inflammation-related diseases at higher rates and at younger ages, than HIV-negative individuals. Depression also raises the risk for inflammatory-related disorders, regardless of HIV status, and both women and PLWH suffer from depression at a greater frequency than the general population. Recently, appreciation has grown for the contribution of persistent immune activation and inflammation to non-AIDS pathologies in PLWH who are on adequate ART. However, the perpetuating force behind sustained inflammation and depression in otherwise healthy PLWH has not been identified. The interaction of the HIV-impacted immune system with the hypothalamic-pituitary-adrenal (HPA) axis may lead to pervasive inflammation. One of the functions of the main effector of the HPA axis,

glucocorticoids (CORT), is to serve as the body's endogenous anti-inflammatory agent. Thus, in an environment where CORT is hypoactive, inflammation would flourish. A combination of rodent models, investigation in nonhuman primates, and human investigation will be used to illustrate the critical role of interactions between inflammation and the HPA axis in both peripheral inflammation and neuropsychiatric function with particular emphasis on the relationship between the HPA axis, depression, and inflammation within the context of HIV.

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Identification of HIV-1 Tat genetic polymorphisms associated with neurocognitive impairment and CNS compartmentalization

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The current studies seek to identify and characterize genetic sequence variation within HIV-1 Tat on the basis of neurocognitive impairment and anatomical source. HIV-1 Tat sequences were obtained from the Drexel Medicine CNS AIDS Research and Eradication Study (CARES) Cohort as well as from autopsied brain tissue regions obtained from the National NeuroAIDS Tissue Consortium (NNTC). Sequences acquired from the Drexel Medicine CARES Cohort were amplified from PBMCs, while NNTC samples were amplified from six regions of the brain in addition to the spleen. Tat nucleotide sequences were translated and aligned to the HXB2 HIV-1 reference genome in order to compare sequence similarity across anatomical compartments and degree of neurocognitive impairment. Aligned sequence populations were compared across all positions of Tat in order to assess the diversity of amino acid usage at each position, as well as to assess enriched polymorphisms between peripherally- and CNS-

derived sequences and sequences derived from non-cognitively impaired versus cognitively-impaired patients. Statistically enriched polymorphisms were structurally modeled computationally with respect to binding the NMDA receptor and P-TEFb complex. Overall, these analyses have resulted in the identification of distinct patterns of amino acid usage between brain and PBMC-derived Tat sequences, as well as Tat sequence variants specific for patients in the absence or presence of neurocognitive impairment which may prove useful in further characterization of HIV-1 pathogenesis.

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HIV-1 Tat and methamphetamine mediated synergistic expression of IL-6 in astrocytes involves PI3K/Akt and NF-κB signaling pathways

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HIV-1 Tat has been implicated in HIV-associated neurotoxicity through production of pro-inflammatory cytokines and oxidative stress in the CNS. Recently, we have shown that methamphetamine causes up-regulation of various pro-inflammatory cytokines. However, combined effect of methamphetamine and HIV-1 Tat on the production of pro-inflammatory cytokines is not known. The present study was undertaken to determine the cumulative effect of methamphetamine and HIV-1 Tat on IL-6 production in astrocytes. The IL-6 expression was measured at different times (3 h, 6 h, 12 h, 24 h and 48 h). Our results clearly demonstrate synergy between HIV-1 Tat and methamphetamine at both mRNA and protein levels. The peak IL-6 expression at mRNA and protein were found to be at 6 and 48 hours, respectively. The underlying signaling mechanism(s) in the up-regulation of IL-6 were identified using pharmacological inhibitors for intermediate steps involved in PI3K/Akt and NF-κB pathways. Appropriate controls were used in the experiments and the effects of pharmacological inhibitors were observed at both mRNA and protein levels. This study therefore provides novel insights into the interaction of HIV-1 Tat and methamphetamine on the synergistic expression of IL-6 by astrocytes along with the

underlying mechanisms with possible role in neuroAIDS. This study also provides information on novel targets that could be utilized for therapeutic potential.

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ALCOHOL REGULATES HIV-1-MEDIATED ASTROCYTE INFLAMMATORY RESPONSES VIA cPLA2 SIGNALING PATHWAY

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Alcohol (EtOH) abuse and HIV-1 remain significant public health problems. Globally, drinkers have approximately 70-77% higher risk of HIV-infection than non-drinkers. The prevalence of alcohol abuse among HIV-positive individuals has been estimated to be between 29-60% in the United States. Many studies showed that neurodegeneration in alcohol abusers include exacerbated neuroinflammation and oxidative damage. However, how EtOH regulates HIV-1-induced astrocyte neuroinflammation is unknown. Thus, we explored mechanism(s) involved in alcohol-mediated activation of human astrocytes with HIV-1 and subsquent alterations in their inflammatory functions. Alcohol exposure altered the morphology of astrocytes, proinflammatory responses and induced cytotoxicity in a dose-dependent manner. Time-depended changes were also evaluated. Alcohol and HIV-1 co-treatment decreased cell viability and proliferation, while increasing apoptosis and mitochondrial depolarization. Alcohol and HIV-1 together increased the levels of proinflammatory molecules, IL-1β, TNF-α, CXCL8, TIMP-1 and more importantly, arachidonic acid, known to be downstream of cPLA2. Consistent with this observation, phospho-cPLA2 levels were augmented in HIV-1 and EtOH co-treatment as compared to HIV-1 or EtOH alone. COX2 was upregulated as measured by real time PCR and western blot, whereas co-treatment of HIV-1 and EtOH decreased CYP2E1 levels as compared to EtOH alone. In summary, our results demonstrate that EtOH-mediated astrocyte inflammation and cytotoxicity in context of HAND occurs via cPLA2 signaling.

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A Novel Role of Proline Oxidase-mediated ROS generation in HIV-1 Envelope Glycoprotein gp120 Induced Neuronal Autophagy

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Proline oxidase (POX) is a mitochondrial enzyme that catalytically converts proline to pyrroline-5-carboxylate. This catabolic conversion generates reactive oxygen species (ROS) that triggers autophagy and apoptosis. The goal of this study was to elucidate a role of POX in HIV-1 associated neurotoxicity that is the driver of HIV-1 associated neurological disorders (HAND). HIV-1 envelope glycoprotein-gp120 is a viral neurotoxic factor that plays critical roles in HAND pathogenesis. However, the underlying mechanism of gp120-mediated neurotoxicity remains elusive. This study demonstrates a functional role of POX in gp120 induced neurotoxicity. Treatment of SH-SY5Y neuroblastoma cells with gp120 induced POX expression and catalytic activity in a dose dependent manner. Concurrently, gp120 treatment also resulted in a time-dependent increase in ROS levels in these neuronal cells. Our data suggests that autophagy is the immediate effect of increased ROS presumably for neuronal protection against the toxic effects of gp120. However, sustained accumulation of ROS over time induced neuronal apoptosis as measured by increased annexin-V/ propidium iodide staining. A functional role of POX in gp120 mediated neurotoxicity was examined by inhibition and over-expression studies. Inhibition of POX activity by a competitive inhibitor-dehydroproline, decreased ROS levels concomitant with reduced neuronal autophagy. Conversely, overexpression of POX in neuronal cells activated ROS-dependent autophagy. Collectively, our results demonstrate a novel role of POX as a stress response regulator.

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Exercise protects against methamphetamine-induced neurotoxicity in the hippocampus

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While no effective therapy is available for the treatment of methamphetamine (METH) induced neurotoxicity, behavioral interventions, including aerobic exercise, are being used to improve depressive symptoms, anxiety, and substance abuse outcomes. The present study focuses on the effect of exercise on METH-induced neurotoxicity in the hippocampal dentate gyrus (DG) in the context of the blood-brain barrier (BBB) pathology. We administered METH or saline (vehicle) three times per day for 5 days with an escalating dose regimen at 3 h intervals. One set of mice were sacrificed 1 day post last injection of METH and the remaining mice were divided into two major groups: a) the exercise group and b) the sedentary group. After the chronic METH administrations, the expressions of tight junction (TJ) proteins were decreased in the hippocampus. Importantly, BBB permeability was significantly increased and remained elevated even 20 days after the withdrawal of METH in the METH-induced mice. Moreover, neural differentiation was significantly decreased in METH-exposed hippocampal DG, suggesting impaired neurogenesis. Most importantly, voluntary exercise protected against this effect, enhanced the protein expression of occludin, a TJ protein, and inhibited induction of inflammatory cytokines in METH-exposed mice. These results suggest that exercise can reverse METH-induced neurotoxicity by increasing TJ protein expression, which protecting against the BBB disruption-related microenvironmental changes in the hippocampus.

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Ageing and NeuroAIDS: Decoding the Synapse

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Methamphetamine (METH) is a potent psychostimulant with its abuse increasing at an alarming rate globally. METH, which has become the illicit drug of choice among the youth due to its ease of availability and long lasting stimulatory effects, has been documented to increase the severity and progression to NeuroAIDS in HIV-1 infected individuals. Adding further complexity to this is the rapid cognitive decline resulting in

accelerated aging of the brain in young HIV+ subjects dependent on METH. Our long-term objective is to advance our understanding of the neural processes underlying the synergy of HIV and METH in young versus old HIV Tg rats with emphasis on the synapse. We hypothesize that HIV in conjunction with chronic METH abuse induces age specific synaptic changes in the brains of young vs old HIV Tg rats. Our preliminary studies are aimed at evaluating change in expression of synaptic markers such as synaptophysin, PSD95 and vGLUT1 in the brains of young vs old HIV Tg rats dependent on METH by immunohistochemistry. In addition changes in dendritic and neurite length between the two age groups will be assessed. These preliminary data will be presented at the conference. Future goals include mining the synaptic proteome to identify synaptic signatures that could further shed new light into understanding the underlying bases by which HIV and METH induce neurocognitive deficits in the young vs old Tg rats. These findings could have relevance for future development of therapeutic interventions aimed at mitigating HIV and METH mediated neurotoxicity.

Cocaine induces endoplasmic reticulum stress-mediated activation of autophagy in astrocytes: Implications for astrogliosis

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Cocaine abuse is known to induce inflammation thereby augmenting the pathogenesis of neurodegeneration. Recent study from our lab reveals a link between autophagy and microglial activation. Herein we explored the cocaine-mediated autophagy by determining the protein expressions of autophagy markers such as beclin1, ATG5, LC3-II and p62 in human A172 and primary astrocytes in a dose- and time-dependent manner. Cocaine treatment resulted in increased formation of LC3 puncta in both human astrocytes expressing either endogenous LC3 or overexpressing GFP-LC3. Intriguingly, cocaine induced the autophagy through upstream activation of ER stress proteins such as PERK, IRE1alpha, and ATF6 in a dose- and time-dependent manner. Cocaine-mediated autophagy in astrocytes was blocked by autophagy inhibitors, 3-MA and wortmannin, as well as by Becn1 siRNA, thereby confirming the involvement of ER

stress as an upstream event in the pathway. Additionally, salubrinal, an ER stress inhibitor, was also able to protect the astrocytes from cocaine-mediated autophagy. In summary, cocaine induced astrocyte activation via the ER stress and autophagy pathways. Pharmacological and genetic silencing approach confirmed the association of ER stress-mediated autophagy activation and GFAP expression in cocaine-treated astrocytes. Increased GFAP expression correlated with increased expression of inflammatory markers such as TNF, IL-1B, and IL-6. Therefore, intervening ER stress and autophagy signaling would provide promising therapeutic targets for cocaine-mediated neuroinflammatory diseases.

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The effect of methamphetamine self-administration on gut-mucosal barrier integrity in adult HIV-1 transgenic rats

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Methamphetamine (meth) abuse is a serious health problem in the HIV-infected community. Meth-abusing HIVpos individuals exhibit greater morbidity and mortality than HIVpos individuals who do not abuse meth or HIVneg meth abusers. The mechanisms that underlie this exaggerated pathology remain unclear. Gut-mucosal barrier dysregulation and break down are consequences of HIV infection and inflammation. It is known that meth is pro-inflammatory and disrupts the blood-brain barrier, but the capacity of meth to damage the intestinal barrier is unknown. To enhance understanding of the gut-mucosal consequences of meth abuse and HIV/AIDS, we are studying a rodent model of human HIV infection on antiretroviral therapy, HIV-1 transgenic (Tg) rats. Male non-Tg and Tg rats self-administered meth 2hr daily for 21 days; controls were saline-yoked. On day 22, we measured urine excretion of orally gavaged sugars (sucrose, mannitol, lactulose and sucralose), and intestinal permeability was determined by a change in the sucralose/lactulose ratio. A two-way ANOVA revealed an effect of genotype ($p=0.001$), but not meth ($p=0.25$), indicating compromised gut-mucosal barrier function in Tg rats. To determine if leaky

gut correlated with a marker of microbial translocation, we measured serum levels of lipopolysaccharide binding protein (LBP). In meth-treated Tg rats, there was a trend for a positive correlation between the sucralose/lactulose ratio and serum LBP (p=0.058). These studies indicate that gut-mucosal barrier integrity is impaired in Tg rats, and this effect may be exacerbated by meth.

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MicroRNA-155 regulates SAMHD1 facilitated HIV restriction in astrocytes

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Astrocytes do not support productive infection of HIV, unlike microglia and brain macrophages. The exact mechanisms governing the possible replication blocks and the establishment of HIV reservoir in astrocytes, however, are yet to be delineated. Recently identified restriction factor, SAMHD1, has been shown to restrict HIV infection in resting CD4+ T cells, monocyte derived dendritic cells and macrophages by depleting dNTPs. Moreover, certain microRNAs (miRNA) have been implicated in HIV restriction in resting CD4+T cells. However, the role of SAMHD1 activity and miRNA expression in HIV-1 restriction activity in astrpcytes are unknown. We hypothesized that increased SAMHD1 expression/activity in astrocytes hampers retrovirus reverse transcription, which in turn leads to non-productive infection, and host miRNAs regulate SAMHD1 expression. We saw that astrocytes have higher level of SAMHD1, silencing of which relieves this restriction and low level of miR-155 compared to microglia. miR-155, by suppressing the induction of p21, regulate the phosphorylation of SAMHD1. Here we report for the first time that SAMHD1 is responsible for the HIV restriction in astrocytes and miR-155 regulates SAMHD1 expression. In conclusion, our data demonstrate that if optimal combinations of both of these could be effectively delivered to astrocytes along with HAART in in-vivo condition, eradication of CNS viral reservoirs may be possible, opening novel avenues to improve current strategies to trigger reactivation of the latent CNS reservoir in patients.

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Differential effect of human dopamine transporter site-directed mutations on HIV-1 Tat-induced inhibition of dopamine transport

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HIV-1 Tat protein disrupts dopamine (DA) neurotransmission by inhibiting DA transporter (DAT) function in HIV-1 positive individuals, leading to the development of HIV-1 associated neurocognitive disorders. We have demonstrated that Tat inhibits DAT through a protein-protein interaction and allosteric modulation mechanism. In the present study, we computationally determined the binding mode of human DAT (hDAT) with Tat and predicted five binding residues of hDAT for Tat. The role of Tyr470, Tyr88, Lys92, Asp206, and Asp381 of hDAT in Tat-induced inhibition of DA transport was characterized by site-directed mutagenesis. Y470H, Y470A, Y88F, and K92M but not Y470F attenuated Tat-induced inhibition of DA transport, implicating a structure and function relationship between Tat and DAT. Compared to wild-type (WT) hDAT, the Vmax of [3H]DA was reduced in Y470H, K92M, D381L, and Y470A but not Y88F, D206L, or Y470F without changes to the Km. Changes in Vmax were not accompanied by changes in DAT surface expression. In addition, only Y88F and K92M enhanced IC50 values for DA inhibition of [3H]DA uptake but decreased IC50 values for cocaine and GBR12909 inhibition of [3H]DA uptake, suggesting that the residues are critical for substrate and these inhibitors. These mutants distinctly alter zinc-induced modulation of either [3H]DA uptake or [3H]WIN35,428 binding relative to WT hDAT, suggesting that these mutations differentially modulate transporter conformation transitions. Our studies provide structural and mechanistic insights into identifying target residues on hDAT for Tat binding.

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Effect of naphthalene, an important cigarette smoke constituent, on cytochrome P450 and oxidative stress pathways in U937 monocytic cells: Implications with HIV pathogenesis

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Smoking, which is highly prevalent in HIV population, is known to exacerbate HIV pathogenesis, perhaps through oxidative stress pathway. Naphthalene (NP), a key polycyclic aromatic hydrocarbon of tobacco smoke, has been designated as a carcinogen and stimulant of oxidative stress through cytochrome P450 (CYP) pathway in lungs. This study was designed to examine the effect of NP on CYP and oxidative stress pathways on HIV model system, namely monocytic cells, which may lead to decreased response to antiretroviral therapies (ART) and increased HIV replication. Initially, we examined the role of NP exposure on the expression of CYP and antioxidant enzymes (AOE) in U937 monocytic cells. NP exposure from 6-24 hours differentially altered the mRNA expression of NP-metabolizing CYP1A1 and ART, especially protease inhibitor (PI)-metabolizing CYP3A4 at 100 nM and 1 µM. However, it did not alter the mRNA expression of AOE, superoxide dismutase 1 (SOD1) and catalase. Moreover, the western data demonstrated a time- and concentration-dependent increase in the protein expression of all four proteins. We are now in the process of determining NP-induced enzyme activities of CYP and AOE, as well as, oxidative stress using DNA damage and glutathione assays and cellular toxicity using MTT and LDH assays. Further, we will perform detailed mechanistic studies to examine the role of CYP and oxidative stress pathways in NP-mediated HIV replication and ART efficacy in HIV-infected primary macrophages. The study has clinical significance in terms of treating HIV-infected smokers more effectively.

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Exposure to phenanthrene, an important constituent of tobacco, and possible role of cytochrome P450 and oxidative stress in monocytic cells: Implications for HIV pathogenesis

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Tobacco smoke aggravates HIV-1 pathogenesis and leads to decreased responses to antiretroviral therapy (ART). However, the mechanism by which smoking enhances HIV pathogenesis is yet to be explored. Phenanthrene (PH), a major chemical component of tobacco and a causative agent for lung cancer, requires metabolic activation through cytochrome P450 (CYP) to exert its tumorigenic actions. In current study, we explored the effect of PH on CYP and oxidative stress pathways in an in vitro HIV model system, U937 monocytic cell line. First, we determined the effect of PH on the mRNA expression levels of CYP and antioxidant enzymes (AOEs). When cells were exposed to 1 µM PH, they showed 2-3 fold increase in the mRNA of ART-metabolizing CYP3A4, but not PH-metabolizing CYP1A1. No significant changes were observed in mRNA expression of AOE superoxide dismutase 1 (SOD1) and catalase. Furthermore, our western results demonstrated no significant alterations in the levels of CYP1A1 and CYP3A4 proteins, but a decreasing trend in SOD1 and catalase proteins at both 12 and 24 hours. Currently, studies are underway to explore the influence of PH on the activities of CYP and AOEs, NP-induced oxidative stress by measuring DNA damage and glutathione levels, and cellular toxicity. Finally, a comprehensive characterization of CYPs and AOEs in HIV-infected macrophages will be performed to assess the role of PH on HIV replication and efficacy of ART. The outcomes from the present work are clinically significant in the context of determining the optimal dosing regimen for HIV positive smokers.

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Effects of cigarette smoke condensate on HIV pathogenesis

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Cigarette smoking is known to exacerbate HIV pathogenesis. However, its underlying mechanism is poorly known, except the fact that oxidative stress is associated with smoking mediated HIV replication. Since cytochrome P450 (CYP) pathways are known to cause smoking mediated oxidative stress and lung cancers, we hypothesize that CYP pathways contribute to smoking mediated HIV pathogenesis. Towards this, we have recently shown that CYP2A6-mediated nicotine metabolism increases oxidative stress in monocytic cells, and this finding is associated with increased oxidative stress and viral replication in HIV-infected smokers than nonsmokers. In this study we show the contributions of CYP1A1 and CYP1B1 on cigarette smoke condensate (CSC)-mediated HIV replication. CSC contains polycyclic aromatic hydrocarbons (PAHs), which are known to be metabolized by CYP1A1 and CYP1B1 producing oxidative stress and cell toxicity. Our results showed that CSC increases HIV replication in HIV-infected primary macrophages, which is consistent with increased levels of CYP1A1 (51-fold) and CYP1B1 (19-fold) in HIV-infected primary macrophages. To investigate the contribution of specific PAHs and contribution of CYP-mediated oxidative stress and toxicity further, we used U937 monocytic cells. The results showed that CSC induces CYP1A1 and generates reactive oxygen species (ROS) that leads to oxidative DNA damage and cellular toxicity. Further, among PAHs (benzo(a)pyrene, naphthalene, and phenanthrene), benzo(a)pyrene primarily contributed to the increased CYP expression and oxidative stress in U937 cells.

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Chronic effects of alcohol and/or antiretroviral drugs on monocytes/macrophages: Role of cytochrome P450, efflux transporter, and antioxidant enzymes

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Alcohol drinking has been identified as a major impediment to the success of antiretroviral therapy (ART) due to undesirable effects of alcohol on efficacy and toxicity of ART. Our previous study has shown that acute exposure to alcohol causes cellular toxicity through cytochrome P450 (CYP) 2E1-mediated oxidative stress in monocytic and astrocytic cells. We have also shown that alcohol alters the interaction of ART-metabolizing CYP3A4 with protease inhibitors (PI). This suggests that alcohol would change the bioavailability of ART drugs causing an altered drug efficacy and increased drug toxicity. This study was designed to examine the chronic effects of alcohol and/or PI (darunavir and ritonavir) on cellular toxicity through CYP- and efflux transporter-mediated pathways. The results showed that chronic exposure of alcohol and ART (12-day) alone (~2-fold) and in combination (~5-fold) downregulated the expression of CYP3A4. In addition, the level of PIs' efflux transporter ABCC1 was also reduced (~25%) by chronic exposure to alcohol and alcohol+PI. Decreased levels of both CYP3A4 and efflux transporter would significantly build-up the concentration of these PIs ultimately leading to drug toxicity in monocytes. Further, we have shown that the levels of most antioxidant enzymes (AOEs) are either reduced or unaltered, suggesting a compromised defense system against drug-mediated toxicity in the cells. Currently, we are in the process of determining the impact of these findings on alcohol and/or PI mediated cellular toxicity and viral replication in HIV-infected macrophages.

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Polydrug nanocarriers to treat opiate addiction and reduce HIV exNef-associated neuropathogenesis.

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HIV incidence and neurocognitive impairments continue to increase among intravenous drug users such as heroin addicts. In fact opiates have been shown to enhance HIV induced neuropathology. Several studies report that the expression of the HIV

accessory protein, Negative factor(Nef) in cells of the central nervous system (CNS). Nef has been shown to be released from HIV-infected or nef-transfected cells in exosomes and that these Nef+ exosomes (exNef) may be associated with neuropathogenesis. Currently there are no therapeutics that directly address HIV neuropathogenesis and opiate addiction. Treating HIV infection in opiate abusers is challenging due to the increase rate of disease progression and low adherence to anti-HIV therapy (due to the addiction). Here we have developed a nanomedicine-based therapeutic to directly block opiate receptors and inhibit HIV Nef neurotoxicity. Magnetic nanocarriers with a cocktail consisting of CTOP, a μ-antagonist and miRNA 29, a microRNA targeting Nef (MNP_CTOP&miR29), and a Nef peptide adsorbed to the surface were used in the mixed cell in vitro BBB. We monitored BBB integrity and permeabilty as measured by TEER and Dextran-FITC transport assay,respectively. Opiate mediated effects on the BBB, HIV Nef expression, and neurotoxicity were significantly reduced by MNP_CTOP&miR29+Nef peptide. We provide a proof-of-concept using extracellular miRNA and opiate receptor antagonist that HIV-induced neuropathogenesis can be blocked while treating opiate addiction.

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A subset of CD8+ T cells (CD4DIMCD8Bright Cells) control HIV infection in the brain

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CD8+ T cells are prominent in brains of humans and macaques with HIV-E and SIV-E, respectively, yet their role in HIV control in the CNS is not entirely clear. A subset of CD8+ T cells, CD4dimCD8bright T cells (referred to as DP cells), is enriched in anti-HIV responses and in blood of HIV+ Long Term Non-Progressors. We evaluated the ability of DP T cells to control HIV infection in the brain in comparison to CD8 single positive (SP) T cells as modeled in HIV+ NOD/SCID/IL-2r-/- mice reconstituted with human PBMCs (NSG-huPBMCs). We demonstrate that CD8SP (40% of CD3+ T cells) and DP T cells (15% of CD3+ T cells) are found in the brains of HIV+NSG-huPBMCs mice. DP

cells harbor HIV, but interestingly, at three weeks post-infection, greater than 90% of CD4SP cells were depleted, while 25% of DP cells survived. Intracranial injection of CD8SP cells into the brain of NSG-huPBMCs mice induced DP expression by 10-fold indicating that the microenvironment of the brain is conducive to the DP phenotype. Both CD8SP and DP cells continue to proliferate in the CNS. One week after injection 90% of DP cells and 80% of CD8SP cells were Terminal Effector Memory (TEMRA) cells (CCR7-CD27-CD28-CD45RA+). Importantly, increased percentage of DP cells inversely correlated with expression of HIV-gag mRNA (R=-0.61, ¤0.001) but no such correlation existed between CD8SP and HIV-gag mRNA (R=-0.23, ¤0.3). Lastly, we show the presence of DP T cells in brains of HIV-infected individuals. Collectively, our studies point to DP cells as a subset of CD8+ T cells responsible for HIV control in the CNS.

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Astrocytic HIV-1 Tat Protein causes Inflammation in Peripheral Organs

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Astrocytes are infected by HIV-1 typically resulting in a latent infection where only early viral proteins are produced. Since combination antiretroviral therapy targets viral replication, early protein production by astrocytes may still occur and could contribute to the ongoing HIV-1 associated neurocognitive disorders in treated patients. Tat is an early HIV-1 protein that is known to be a potent neurotoxin. Previous work in our lab developed a model of learning impairment by expression of Tat in astroctyes in the hippocampus of Sprague Dawley rats. We have extended this work to gain a better understanding of the toxicity to the brain and other organs caused by Tat in this model. Rats were infused with astrocytes transfected to produce Tat or GFP (control). Seven days after surgery, the rats were sacrificed and the brain, colon, ileum and lung tissues were collected. Tissues were used for histological analysis and immunoassays. We did not find neuronal damage (by Nissl staining) caused by Tat treatment. Lung pathology

was suggestive of acute and chronic interstitial pneumonitis (ex. increased lymphocytes and neutrophils). Microscopic and histological analyses showed significant damage to the colon and increased number of Peyer's patches in the ileum in the Tat-treated group. These findings suggest that astrocytic production of the neurotoxin Tat contributes to a systemic inflammatory response.

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Differing roles of autophagy in HIV-associated Neurocognitive Impairment and Encephalitis with Implications for Morphine Co-exposure

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Autophagy disruption has been implicated as a contributing factor in neurodegenerative diseases. We investigated the role of autophagy in HIV-infected subjects with neurocognitive impairment (NCI) ± HIV encephalitis (HIVE), many of which had a history of substance abuse, using post-mortem brain tissues to determine whether differences in autophagy related factors may be associated with NCI or NCI-encephalitis. Using qRT-PCR, we detected significant differences in gene expression levels with SQSTM1, LAMP1 higher in HIV-infected subjects without NCI while ATG5, SQSTM1 were then lower with NCI and ATG7, SQSTM1 were higher in NCI-HIVE. Immunohistochemical labeling of these autophagy associated proteins in Iba-1-postive microglia showed higher immunoreactivity in the NCI and NCI-HIVE groups with more focal vs. diffuse patterns of expression in the NCI-HIVE group. Furthermore, microarray analysis from these same subjects found significantly higher levels of LAMP1 in NCI-HIVE compared to uninfected subjects in the basal ganglia. We tested the effect of supernatant from HIV-1-infected microglia and HIV-1 Tat protein in combination with morphine in vitro and found both significant inhibition of autophagic flux and reduced dendrite length for neurons. These results suggest that autophagy genes and corresponding proteins may be differentially regulated at the transcriptional, translational, and post-translational

levels in the brain during various stages of the HIV disease and that infected individuals exposed to morphine may have more severe NCI than those without opioid use.

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GRP78 potential marker of Neurocognitive Impairment in HIV Infected Polydrug Users

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PURPOSE: Due to the high prevalence of neurocognitive impairment (NCI) in HIV infected and drug abuse patients worldwide, there is an urgent need to study the synergism between these disorders and their impact on the immune response. We hypothesize that HIV infected polydrug users (PDU) have a greater impact on NCI, a negative effect on T-lymphocyte protein and proinflammatory cytokine expression. To test this hypothesis, we will 1) identify NCI in polydrug addicts participants with and without HIV infection, 2) analyze the cytokine profiling and protein expression in HIV+/HIV-PDU. METHODS: NCI was measured by psychological analysis in HIV+/HIV-PDU. Alterations on protein expression were detected through a proteomic approach and cytokine profiling was achieved by means of flow cytometry. RESULTS: The sample population was distributed: 10 HIV+PDU+, 10 HIV-PDU+. HIV-PDU+ participants had higher NCI and expression of proinflammatory cytokines as compared to HIV+PDU+. Proteomic data showed that ER stress marker GRP78 was downregulated in NCI participants as compared to non NCI participants. Moreover, GRP78 expression diminished with HIV infection. CONCLUSIONS: Our findings showed that the downregulation of GRP78 may be a pontential biomarker to identify NCI in HIV positive PDU This study will contribute to a deeper understanding of the cellular and molecular mechanisms linking drug addiction and HIV infection.

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Characterization of on Demand Delivery of Fluorescent Magnetic Nanoparticle (MNP) Targeted Towards the Brain

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Present Active Antiretroviral Therapy (ART) has significantly improved disease pathology of HIV infection. However, since many drugs have less penetrance into the brain due to Blood Brain Barrier, development of an active targeted drug delivery system is essential to increase the efficacy of drug targeting. In this regard, we developed a fluorescent dye conjugated magnetic nanoparticle (MNP) bound to anti-HIV drug azidothymidine 5'-triphosphate (AZTTP) (MNP-AZTTP), encapsulated into liposomes for targeting to mouse brain. In vitro drug release 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 up to 2hrs. 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 over free drugs. In vivo analysis indicated that within 2hr of injection the fluorescent MNP-AZTTP reached the brain. In vivo toxicological analysis also showed there was no immediate toxicity in mice with MNPs. This novel formulation will be able to on demand deliver multiple anti-HIV drugs more efficiently to rodent brain in a stipulated time.

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HIV-1 Tat induces mitochondrial dysfunction in neurons

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In late stages of HIV infection a subset of individuals develops HIV associated neurocognitive disorders, characterized by dendritic pruning, synaptic abnormalities, and neuronal apoptosis. Little is still known about the molecular mechanisms by which HIV induces this degeneration. HIV protein Tat is known to promote synaptic simplification in vitro and in vivo. Moreover, Tat is suspected to disturb mitochondrial function. Therefore, we examined whether Tat impairs mitochondrial homeostasis in neurons. Primary rat cortical neurons were exposed to 100nM Tat for various durations. Mitochondria membrane potential loss occurs at 4h and persists up to 72h.This deficit was accompanied by their somal accumulation (live cell imaging) and fragmentation (measured by 3DSTORM analysis). These defects in mitochondria could be due to impaired fusion-fission machinery such as over-activity of dynamin-related protein 1 (Drp1). In fact in neurons exposed to Tat we observed time-dependent overexpression of Drp1 (began 4h after Tat exposure). Fragmented and impaired mitochondria must undergo mitophagy. Using LC3 levels as a marker of mitophagy we established no difference between Tat treated and control cells. With these findings we speculate Tat exposure in neurons leads to damaged mitochondria accumulation. These faulty mitochondria can further deplete neurons of ATP, increase ROS production, and ultimately lead to neuronal apoptosis (as published previously by Rozzi et al, 2014). Further investigations into the mechanism of this toxicity are needed to identify therapeutic interventions.

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Effects of stress and stress hormones on cognition and brain function in women with HIV

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HIV-infected women report high rates of exposure to acute and chronic stressors such as those resulting from early childhood trauma. These stressors can lead to profound and sustained negative physiological and psychological consequences including cognitive deficits and brain dysfunction. This presentation focuses on the role of stress and stress hormones on cognition and brain function in women living with HIV. We will review our epidemiological studies examining associations between stress (perceived

stress, post-traumatic stress), anxiety, and cognition in the Women's Interagency HIV Study (WIHS), the largest, longitudinal, multisite study of HIV-infected and at-risk HIV-uninfected women (N~1500). To date, we have demonstrated that higher levels of perceived stress and severe anxiety are associated with deficits in verbal learning and memory in HIV-infected, but not HIV-uninfected women. To elucidate these behavioral findings in HIV-infected women, we examined relationships between perceived stress and brain structure, focusing on regions critical for verbal memory. Results indicated that atrophy in the prefrontal cortex was associated with stress-related deficits in verbal memory in HIV-infected women. The time course of these brain changes in relation to memory deficits has yet to be elucidated, but the magnitude of the volumetric differences suggests a prolonged vulnerability due to chronic elevations in perceived stress. This line of work provides a foundation for future mechanistic and treatment studies aimed at improving cognition among women living with HIV.

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Antibody blocking of CLEC12A delays the course and attenuates EAE severity by impairing myeloid cell CNS infiltration: A potential new therapy for multiple sclerosis

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The mechanism of dendritic cell (DC) recruitment across the blood brain barrier during neuroinflammation has been the least explored amongst all leukocytes. For cells of myeloid origin, while integrins function at the level of adhesion, the importance of lectins remains unknown. We pursued the study of a highly specific aspect of DC biology, namely the expression of surface C-type lectin receptors (CLRs) for their importance in

adherence and transmigration across the blood-brain barrier in response to CCL2 chemotaxis. CLEC12A, an immunoreceptor tyrosine-based inhibitory motif (ITIM) known to recruit Src homology region 2 domain-containing phosphatase (SHP)-1/2, was subsequently identified as one such a target on DCs. SHP-1/2 phosphorylation was further found to facilitate the actin polymerization on DCs that express the WASP Interacting Protein (WIP). To test function of CLEC12A in an animal model of multiple sclerosis (MS), we administered an antibody to block CLEC12A that significantly ameliorated the progression and relapse of experimental autoimmune encephalomyelitis (EAE) in mice through inhibition of demyelination and myeloid cell infiltration into the CNS tissue. Correspondingly, a restoration of DC numbers in the periphery along with decrease in TH17 phenotype within CD4+ T cells was also seen. These studies reveal the utility of a DC-specific mechanism in designing new therapeutics for MS.

Antibody blocking of CLEC12A delays the course and attenuates EAE severity by impairing myeloid cell CNS infiltration: A potential new therapy for multiple sclerosis

Sagar, D, MS[1], Singh, NP, Ph.D.[2], Huang, X, Ph.D.[4], Ginwala, R, MS[1], Philip, R, Ph.D.[4], Nagarkatti, M, Ph.D.[3], Nagarkatti, P, Ph.D.[2], Khan, ZK, Ph.D.[1], Jain, P, Ph.D.[1]; [1]Department of Microbiology and Immunology, Drexel University College of Medicine, Philadelphia, PA, 19102 United States. [2]Department of Pathology, Microbiology and Immunology, University of South Carolina, Columbia, SC, 29208 United States. [3]Department of Pathology, Microbiology and Immunology, William Jennings Bryan Dorn VA Medical Center, Columbia, SC, 29209 United States. [4]Pennsylvania Biotechnology Center, Immunotope Inc., Doylestown, PA, 18902 United States.

The mechanism of dendritic cell (DC) recruitment across the blood brain barrier during neuroinflammation has been the least explored amongst all leukocytes. For cells of myeloid origin, while integrins function at the level of adhesion, the importance of lectins remains unknown. We pursued the study of a highly specific aspect of DC biology, namely the expression of surface C-type lectin receptors (CLRs) for their importance in adherence and transmigration across the blood-brain barrier in response to CCL2 chemotaxis. CLEC12A, an immunoreceptor tyrosine-based inhibitory motif (ITIM) known

to recruit Src homology region 2 domain-containing phosphatase (SHP)-1/2, was subsequently identified as one such a target on DCs. SHP-1/2 phosphorylation was further found to facilitate the actin polymerization on DCs that express the WASP Interacting Protein (WIP). To test function of CLEC12A in an animal model of multiple sclerosis (MS), we administered an antibody to block CLEC12A that significantly ameliorated the progression and relapse of experimental autoimmune encephalomyelitis (EAE) in mice through inhibition of demyelination and myeloid cell infiltration into the CNS tissue. Correspondingly, a restoration of DC numbers in the periphery along with decrease in TH17 phenotype within CD4+ T-cells was also seen. These studies reveal the utility of a DC-specific mechanism in designing new therapeutics for MS.

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 exhibits immunodominance. Here, we evaluate CD8 T-cell immune response against this epitope 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 CD8T 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.

Effect of Magneto-electric nanoparticle on deep brain motor coordination activity

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Magnetic and electric fields depending on their intensity and frequency have been shown to exert theranostic significance in several diseases. Recently we have developed a novel multiferroic magneto-electric nanoparticles (MENPs) which possess strong coupling ability of its magnetic and electric fields at body temperature and showed efficient delivery of anti-HIV drug across in vitro blood brain barrier (BBB). This study encompasses potential application of MENPs as safe and effective in vivo drug-carrier for brain targeting. As such, effect of MENPs on brain physiology was examined by motor cognition and homeostasis analysis of mice treated with MENPs. Mice were intravenously injected with In vitro determined non-toxic dose of MENPs and, subsequently, their brain region was exposed to 0.2 Tesla magnetic fields for 2 hr to drive nanoparticles to brain. At 48 hrs post-treatment period, mice were subjected to motor coordination analysis using 2 and 4 mm horizontal bar test and similar results were obtained in MENPs injected and control groups. Similarly, tail-flick assay suggested that MENPs effect on nociceptive stimulation remained unaffected in compare to control. Further glutathione synthase which is associated with normal brain homeostasis was also expressed in hypothalamus at similar levels in injected and

control groups. It was concluded that the proposed dose of MENPs at 10mg/Kg is safe for drug targeting to brain in mice. Moreover, this study establishes aquantitative method for evaluating the effects exerted by MENP-drug nanoformulations on brain functions.

Methamphetamine alters the normal progression by inducing cell cycle arrest in astrocytes

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Methamphetamine (MA) is a potent psychostimulant with a high addictive capacity, which induces many deleterious effects on the brain. Chronic MA abuse leads to cognitive dysfunction and motor impairment. MA affects many cells in the brain, but the effects on astrocytes of repeated MA exposure is not well understood. In this report, we used Gene chip array to analyze the changes in the gene expression profile of primary human astrocytes treated with MA for 3 days. Range of genes were found to be differentially regulated, with a large number of genes significantly downregulated, including NEK2, TTK, TOP2A, and CCNE2. Gene ontology and pathway analysis showed a highly significant clustering of genes involved in cell cycle progression and DNA replication. Further pathway analysis showed that the genes downregulated by multiple MA treatment were critical for G2/M phase progression and G1/S transition. Cell cycle analysis of SVG astrocytes showed a significant reduction in the percentage of cell in the G2/M phase with a concomitant increase in G1 percentage. This was consistent with the gene array and validation data, which showed that repeated MA treatment downregulated the genes associated with cell cycle regulation. This is a novel finding, which explains the effect of MA treatment on astrocytes and has clear implication in neuroinflammation among the drug abusers.

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Effect of hydromorphone on gut barrier function and immunomodulation in the context of DSS induced colitis

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Morphine is considered as gold standard for the management of pain. However, attaining the required degree of analgesia often leads to tolerance involving dose escalation. Recently, focus has shifted to the use of hydromorphone, which is more potent with fewer side effects. Large studies have been conducted to show that morphine treatment can disrupt intestinal epithelial barrier and suppress immune system, which contribute to induction of sepsis. Inflammatory bowel disease (IBD) is a pathological inflammatory condition of the bowel due to gut barrier disruption and altered immune response. The dextran sulfate sodium (DSS) induced colitis is well accepted and widely used model of IBD. There is no concrete study about the effects of hydromorphone on immunomodulation and gut barrier function in the context of DSS colitis. In the present study, wild-type mice (C57BL/6) were treated with hydromorphone Intraperitoneally (2.5-15mg/kg; BID) with or without DSS for 7 days while untreated mice served as control. Our results showed that bacterial translocation and weight loss were significantly greater in hydromorphone treated DSS animals when compared to all other treatment groups. In colonoscopy, hydromorphone treated DSS animals showed marked erythema, friability, and granularity. We also observed on histology, increased disruption of the gut epithelial barrier in hydromorphone treated animals with DSS colitis compared to control mice. All the various parameters consistently demonstrated that exposure to hydromorphone exacerbates the onset and course of colitis in mice.

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Marijuana THC attenuates allogeneic host-versus-graft response and skin allograft transplants through induction of MDSCs

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Immune cells have been shown to express cannabinoid receptors and produce endogenous ligands. However, the role of cannabinoids in transplantation, specifically allograft tolerance, has not been previously investigated. In the current study, we tested the effect of δ-9-Tetrahydrocannabinol (THC) on the suppression of allogeneic host versus graft disease (HvGD) and rejection of skin allograft transplants. We studied HvGD by injecting H-2d splenocytes into H-2b mice and studying the immune response in the draining lymph nodes (LN). THC treatment significantly reduced T cell proliferation and activation in LN of the recipient mice and decreased rejection indicator cytokines, including IL-2 and IFN-γ. THC treatment also increased allogeneic skin graft survival. THC treatment in HvGD mice led to induction of myeloid-derived suppressor cells (MDSCs). Using MDSC depletion as well as adoptive transfer experiments, we found that THC-induced MDSCs were necessary for attenuation of HvGD. Through the use of pharmacological inhibitors and knockout mice for CB1 and CB2 receptors, we found that THC worked preferentially through CB1. Together, our research shows for the first time that targeting cannabinoid receptors may provide novel treatment modality to attenuate HvGD and prevent allograft rejection.

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Sonic hedgehog signaling confers neuroprotection in HIV-1 infected humanized mice.

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HIV-1 Associated Neurocognitive Disorder (HAND) is associated with compromised Blood Brain Barrier (BBB) integrity. In this study, Humanized NOD/scid-IL2R null mice were used to understand molecular mechanism of HIV-1 induced BBB damage and associated neuropathologies with a specific focus on the involvement of Shh signaling. Humanized mice were infected with HIV-1 and followed for up to 10 weeks and observed elevated levels of p24 viral protein and drastically reduced number of CD4+ T

cells. HIV-1 infection also resulted in activation of platelets including enhanced soluble platelet factor (PF)-4 levels as well as formation of Platelet Monocyte Complexes (PMCs). Further, we observed BBB damage as determined by impaired exclusion of Evan's blue dye as well as by elevated levels of S100B (a neuronal protein) in the plasma of infected mice. Immunohistochemistry staining showed reduced synaptic density, neuronal damage, microglial activation and astrocytosis in infected mice. The Shh signaling molecules including Shh and Gli1 were also reduced in the infected mouse brain tissue in association with depletion of tight junction proteins Occludin and Claudin5 in brain endothelial cells. Interestingly, rescue of Shh signaling by administration of Smoothened agonist (SAG) restored the BBB integrity and function and prevented neurologic damages induced by HIV-1. Together, our results suggest a neuroprotective role for Shh signaling in context to HIV-1 infection, underscoring therapeutic potentials of SAG in controlling debilitating effects of HAND.

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Potential role of cytochrome P450 and oxidative stress pathways in alcohol-mediated HIV pathogenesis: observed effects in plasma, monocytes, and exosomes.

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Mild-to-moderate alcohol consumption is known to exacerbate HIV pathogenesis. The objective of this study is to determine the specific effects of mild-to-moderate alcohol use on viral load, oxidative stress, and cytochrome P450 (CYP) in HIV-infected individuals. Human subjects were recruited from four different cohorts; a) HIV negative non-drinkers, b) HIV positive non-drinkers, c) HIV negative mild-to-moderate drinkers, and d) HIV positive mild-to-moderate drinkers. Our results showed an increase in the viral load and alcohol metabolism in HIV drinkers compared to the respective control.

Importantly, HIV-infected individuals and drinkers showed an increase in oxidative stress compared with HIV negative non-drinkers in both plasma and monocytes. The results also showed that the levels of most antioxidants are unaltered, but the levels of CYP enzymes, especially CYP2E1 and CYP3A4 are increased in monocytes from HIV positive patients and drinkers compared to control subjects. Furthermore, we isolated exosomes, which are extracellular nanoparticles secreted from many cell types and are known to play a role in HIV pathogenesis. The results showed an altered level in exosomal proteins of both CYP and oxidative stress pathways in HIV-infected non-drinkers and uninfected drinkers compared to control subjects. We are now in the process of studying the role of specific exosomal proteins derived from HIV-infected and alcohol-treated primary macrophages. The study has clinical significance in terms of developing novel approach (exosomes) to treat HIV-infected drinkers effectively.

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Methamphetamine enhances HIV-1 infection of neural stem cells

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Methamphetamine (METH) abuse and HIV infection often coexist, mostly because of the association of METH abuse with the engagement in high-risk behaviors. Although active METH users were shown to have higher HIV viral load and experience more severe neurological complications than non-users, the direct effect of METH on HIV infection and its link to the development of neurocognitive alternations is still poorly understood. In the present study, we hypothesize that METH enhances HIV-1 infection of neural stem cells by a mechanism encompassing NF-kB/- HIV LTR activation. Such a sequence of events would contribute to deficient neurogenesis and aggravate cognitive deficits of HIV- infected METH users. Cultures of mouse neural stem cell line (NE4C) were infected with EcoHIV/NL4-3, a chimeric HIV-1 that exclusively infects mice. METH at 10, 50 or 100 ÂµM was added 24h before, together, or 24h after EcoHIV infection. To determine mechanism underlying Meth-induced HIV expression, NE4C cells were transfected with different variants of HIV-LTR promoters and then exposed to METH. Our results demonstrate that pretreatment with METH significantly increased EcoHIV

production in a dose-dependent manner. METH treatment induced LTR activation, an effect potentiated by EcoHIV. We also determined that METH activated NF-ÎºB, a key cellular transcriptional regulator of the HIV LTR. These results suggest that METH, by enhancing HIV infection in neural stem cells, may contribute to neurocognitive dysfunction in HIV-infected patients.

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The neuroprotective role of caffeine in LDL-treated human neuroblastoma cell line

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Caffeine, the most widely ingested psychoactive substance in the world, is protective against the development of Alzheimer's disease (AD) as evidenced by its effects on decreasing brain accumulation of amyloid beta (Aβ) and improving cognitive functions. However, the underlying mechanisms remain unclear. We have shown, both in vivo and in vitro, that increased levels of low-density lipoprotein (LDL), a robust exogenous risk factor for AD, increases amyloid precursor protein (APP) internalization, disturbs neuronal endolysosome structure and function, and promotes amyloidogenic processing of APP in neuronal endolysosomes. Here we determined the underlying signaling pathways whereby caffeine protects against LDL-induced amyloidogenic processing of APP in human neuroblastoma cell line (SHSY-5Y) over-expressing wild-type human APP using ELISA, immunoblotting, and immunostaining methods as well as enzyme activity assays. Caffeine concentration-dependently decreased the production of Aβ, decreased LDL-induced elevation of BACE1 protein levels, prevented LDL-induced endolysosome dysfunction as indicated by disturbed proteins levels of the early, late and recycling endosome markers (EEA-1 and Rab 5, 7 & 11) and lysosomal enzyme cathepsin D, and inhibited PI3K/Akt signaling, known to be involved in the regulation of APP processing in endolysosome systems. Our findings suggest that caffeine-induced protection against LDL-induced amyloidogenic processing of APP is correlated with inhibiting PI3K/Akt signaling.

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Single nucleotide polymorphisms (SNPs) in the HIV-1 LTR correlate with clinical disease parameters

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Human immunodeficiency virus type 1 (HIV-1) is continuously mutating, even in well-controlled individuals on HAART. Single nucleotide polymorphisms (SNPs) within the long terminal repeat (LTR) are of particular interest due to its role in regulating viral gene expression. Previous work has shown fixed mutations at particular residues within the LTR have increased with disease severity. Additionally, SNPs within the LTR can have cell-types specific affects, altering the viral fitness and phenotype. Currently, the DrexelMed CNS AIDS Research and Eradication Study (CARES) Cohort in Philadelphia, PA conducted a longitudinal study in 489 HIV-1 seropositive patients to identify LTR signatures from peripheral blood that may aid in characterizing HIV-1 disease progression by clinical parameters such as CD4 + T-cell counts and viral loads. The collection of LTR sequences and extensive clinical parameters on these patients has allowed for cross-sectional and longitudinal analyses of their selection for SNPs during the course of disease. Mutations at position 108, a confirmed COUP/AP1 site, were correlated with patients who had low CD4 + T-cell counts and high viral loads. The in silico prediction algorithm JASPER, predicted significant changes in transcription factor binding affinity when mutated away from the consensus nucleotide. The impact of

this SNP on transcriptional profile was demonstrated by EMSA experiments. However, the functional and cell-type specific affects these SNPs have on HIV-1 pathogenesis have yet to be fully determined.

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HIV infection and/or Heroin use dysregulate the plasma exosome miRNA expression

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Exosomes are small membrane vesicles that are released by most of the cell types, making them abundant in a variety of body fluids, including plasma. Exosomes play a significant role in cell-to-cell communication, immune responses and viral infections. It is recently revealed that exosomes contain functional microRNAs (miRNAs) which can be delivered into recipient cells, exerting biological functions. MiRNAs are also reported to be related to host cell innate immunity and HIV infection. In the present study, we investigated the impact of HIV infection and/or heroin use on the expression of miRNAs derived from plasma exosomes. We found that fourteen miRNAs (223, 20a, let-7a, 17, 16, 195, 29b, 29c, 126, 21, 146a, 125a, 150, 145) were dramatically increased in HIV-infected subjects. Although heroin alone had little effect on the exosomal miRNAs, the up-regulation of miR-150 and miR-17 by HIV was compromised by heroin use. These findings provide direct evidence that HIV infection and heroin use dysregulate plasma miRNAs, which may account for HIV pathogenesis among heroin users.

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Mutation of tyrosine 470 of human dopamine transporter is critical for Tat-mediated allosteric modulation on human dopamine transporter

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HIV-1 Tat protein and cocaine synergistically disrupt dopamine (DA) transmission by inhibiting human dopamine transporter (hDAT), which exacerbates the progression of HIV-1-associated neurocognitive impairment. Our previous study demonstrated that compared to cocaine alone, Tat slowed cocaine-induced dissociation rate of [3H]WIN35,428 binding and increased IC50 value of cocaine inhibiting [3H]DA uptake in wild type (WT) hDAT, indicating that Tat allosterically modulates hDAT. Through the integrated computational modeling and experimental studies, we have identified that mutation of tyrosine470 (Y470H) of hDAT, via changing transporter conformational transitions, alters basal DA transport and attenuates Tat-induced inhibition of DAT. The present study evaluated the role of Y470H in Tat-mediated allosteric modulation of hDAT. SoRI-20041, a novel allosteric modulator of the DAT, increased IC50 value for cocaine inhibiting DA uptake by 33% (IC50 in nM: cocaine, 334 ± 36; cocaine + SoRI-20041, 443 ± 31) in WT hDAT; however, the effect of SoRI-20041 was attenuated in Y470H (IC50 in nM: cocaine, 271 ± 37; cocaine + SoRI-20041, 263 ± 12). Compared to cocaine alone, SoRI-20041 following the addition of cocaine decreased the dissociation rate of [3H]WIN35,428 binding by 43% in WT hDAT and 8% in Y470H, respectively. Collectively, these results indicate that tyrosine470 as a functional relevant residue in hDAT is critical for Tat-mediated allosteric modulation of hDAT. Identifying potential allosteric binding sites in hDAT for Tat in response to cocaine is our important ongoing study.

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Depressive Symptoms Point to New Therapeutic Targets in HIV Infection

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The frequency of major depressive symptoms in HIV+ individuals is nearly double for matched controls, this includes the somatic symptoms of glucocorticoid (GC) resistance; and depressive symptoms are associated with worsened HIV disease. GC resistance is marked by cellular irresponsiveness to the anti-infammatory effects of the stress hormone cortisol. HIV persists in immune cell reservoirs, and one barrier to developing eradication therapies is understanding the factors that lead to viral reservoir expansion and persistence. Our research focuses on examining monocyte expansion, pro-inflammatory cytokine production, and reservoir expansion during depressive symptoms in HIV-infection. We examined potential of CD14+ monocytes to proliferate, produce TNF-alpha, IL-1-beta, and IL-6 in response to IL-3 stimulus despite the presence of cortisol. We found in GC resistance in one HIV+ individual, only during a major depression time point but not a euthymic timepoint. Additionally, we found neuropathological evidence for GC resistance in an central nervous system model of HIV infection, the doxycline-inducible (astrocyte-specific promoter) Tat transgenic mouse (N=12). The evidence suggests that preventing or alleviating GC resistance in HIV-infected individuals is a feasible strategy to reducing long-term viral persistence, central nervous system viral penetrance, and reservoir expansion while viral RNA suppressed. In conclusion we found through a GC resistance assay of ex vivo cultured monocytes, that depressive symptoms associates with inflammation and monocyte expansion in HIV.

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HIV and Morphine: Oxidative Stress Impact Cell Cycle Regulation and Neurodegeneration

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Previous studies have demonstrated that HIV infections and drugs of abuse such as morphine have been identified as risk factors for triggering HIV-1disease progression and neuronal dysfunctions. Microglia is the reservoirs of HIV-1 infection and viral replication in CNS. The synergistic effects of morphine and HIV-1 infection and the potential role of oxidative stress and cell cycle arrest impact on neropathogenesis of HIV-1 infection have not been clearly elucidated. We hypothesize that HIV-1 gp120 with morphine synergistically dysregulate the redox glutathione synthase (GSS), super oxide dismutase (SOD) and glutathione peroxidase (GPx) and cell cycle regulated protein cyclin B, cyclin-dependent kinase 1, cell division cycle 2 (CDK-1/CDC-2) and cell division cycle 25C (CDC-25C) influence on neuronal dysfunction. RNA extracted from human microglia treated HIV-1 gp120 with and without morphine was reverse transcribed and analyzed by quantitative real-time PCR to determine GSS, SOD and GPx gene expression. Cell lysates were analyzed by western blotting to determine redox protein expression in GSS, SOD and GPx and cell cycle regulated proteins cyclin B, CDK-1, CDK-1/CDC-2 and CDC-25C. Our results indicated that HIV-1 gp120 significantly down regulated redox expression and cell cycle regulated protein cyclin B whereas CDK-1, CDK-1/CDC-2 and CDC-25C up regulated and these effects were accelerated by morphine. These results suggest that morphine synergistically inhibit redox expression and subsequently impact cell cycle arrest exacerbating neurodegeneration of HIV-1gp120.

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Post-Transcriptional Regulation of Astrocyte-Tissue Inhibitor Metalloproteinase-1 (TIMP-1) in HAND

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HIV-1 leads to several central nervous system problems together termed as HIV-1-associated neurocognitive disorders (HAND). TIMP-1/MMP imbalance has been

observed in HAND. Astrocytes are major contributors to brain TIMPs and they regulate MMP/TIMP balance. Differential regulation of TIMP-1 in acute versus chronic neuroinflammation is relevant to HAND neuropathogenesis. However, the underlying mechanisms are still being uncovered. Our previous works have shown the neuroprotective role of TIMP-1 via MMP-dependent and independent manners. In this study, we investigated regulation of astrocyte TIMP-1 in HAND. First, microarray analysis was performed to analyze miRNA changes in IL-1β activated astrocytes. 12 miRNAs were significantly increased and four were significantly decreased; seven of those were further confirmed by RT-PCR. The most increased were miRNA 155 and miRNA 146b. For further studies, TIMP-1 3'UTR cloned downstream of Firefly luciferase and miRNA overexpression constructs were used to investigate miRNA-mediated TIMP-1 3'UTR post-transcriptional regulation. Overexpression of miRNA 155 and miRNA 146b altered both Firefly luciferase activity and endogenous astrocyte TIMP-1 levels as measured by ELISA in parallel experiments. Thus, our data suggests that astrocyte-TIMP-1 may be regulated post-transcriptionally by miRNAs during HAND.

Role of autophagy pathways in the neuropathogenesis of HIV-1 clade B/C and the exacerbating effects of drug of abuse

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HIV/AIDS and drug of abuse are interlinked epidemics and numerous studies have shown that drug abuse such as cocaine can increase HIV replication, as well as neuronal toxicity in the presence of HIV infection. The existence of multiple subtypes of HIV worldwide has created new challenges to control HIV infection and associated neuropathogenesis. Both HIV clade C (HIV-C) and HIV clade B (HIV-B) can cause cognitive impairment, with studies indicating clade B being more neuropathogenic than clade C. However, the exact mechanism underlying the differences in the neuropathogenesis by both subtypes remains elusive. Autophagy is a key process involved in proper cellular maintenance and its disruption has been implicated as a contributing factor in neurodegenerative diseases. Therefore, our study focuses on the role of the autophagy pathway as a mechanism regulating the divergence in the

neuropathogenesis of HIV clade B/C in the context of cocaine. Using HIV neurotoxic proteins Tat and gp120 from the different clades in the presence and absence of cocaine, our data indicate that cocaine exacerbates the expression levels of autophagy related genes and the synergy could be attributed to viral protein from the different clades.

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Blood-brain barrier approach to HIV infection and methamphetamine abuse

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The blood-brain barrier (BBB) is the most prominent barrier of the CNS and represents the essential interface between the CNS and the blood stream. The intact BBB is maintained by tight junction (TJ) proteins and is a paramount determinant of brain homeostasis. Disruption of the BBB is frequently observed during drug abuse and brain infections by various pathogens, including HIV. Our results indicate that occludin, one of the major TJ proteins, is decreased upon methamphetamine (METH) exposure and that diminished occludin levels not only lead to the loss of integrity of the BBB but also stimulate HIV replication. The central hypothesis of our research is that cerebrovascular alterations at the BBB level induced by METH have profound impact on establishing and outcome of the brain infection by HIV. This project develops in the following three major research areas: 1) impact of occludin levels on HIV replication in BBB pericytes, 2) impact of disruption of BBB on aberrant neurogenesis of neural progenitor cells resulting in the development of cognitive dysfunction, and 3) behavioral intervention based on exercise for protection against METH and HIV-induced BBB dysfunction and neurocognitive alterations. This research offers a unique perspective on the interactions between METH and HIV via targeting the BBB. However, our results are relevant to other neurodegenerative diseases that have significant cerebrovascular components.

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Development of brain targeted theranostic agent for multimodal imaging and anti-HIV drug delivery

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Delivery of therapeutic agent to brain is hard to achieve due to the presence of blood-brain barrier (BBB). Nanoparticles have been promising material for brain targeting carrier because of their small size range comparable to biological molecules, and the ability to cross BBB by physical force and targeting mechanisms. Magnetic nanoparticles are particularly taking attention for theranostics which is combination of therapy such as drug delivery and imaging within a single platform. In this study, liposome encapsulates magnetic nanoparticles, gold nanorods, and anti-HIV drug was designed as theranostic agent for anti-HIV drug delivery and brain imaging. Magnetic nanoparticles encapsulated in liposome enhanced blood-brain barrier (BBB) transmigration under external magnetic field. Imaging ability of magnetic nanoparticles and gold nanorods was demonstrated for magnetic resonance imaging (MRI) and near infrared (NIR) imaging. Enhancement of transmigration across BBB and imaging ability indicate the feasibility of this liposome as brain targeted theranostic agent.

Tracheo-bronchial Mucociliary dysfunction in HIV patients

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Introduction: Pneumonia continues to be an important comorbidity in HIV infected patients.Recent studies show that the lower respiratory tract is a microbial reservoir in HIV infected individuals similar to that observed in airway diseases characterized by depressed mucociliary clearance (MCC) like Chronic bronchitis and Cystic fibrosis.

While HIV infection is associated with attenuated nasal MCC, not much is known about the tracheo-bronchial MCC in these subjects. HIV infected smokers also have to contend with increased transforming growth factor beta- signaling (TGF-beta) signaling leading to suppressed expression of cystic fibrosis transmembrane conductance regulator (CFTR) a critical protein involved in maintaining optimal MCC. This abstract introduces the role of HIV proteins Tat and gp120 in attenuating components of the MCC apparatus as it relates to understanding the increased rates of bacterial pneumonia in HIV infected patients. Results: Normal Human bronchial epithelial cells (NHBE) exposed to HIV tat demonstrate increased expression of TGF-beta 1. Increased TGF-beta expression and signaling translates to decreased expression of CFTR. In addition, HIV gp120 suppresses baseline ciliary beat frequency (CBF) and this can be restored by beta-2-agonist bronchodilators commonly prescribed in chronic airway diseases. Conclusions: The cumulative effect of HIV tat (on CFTR) and HIV gp120 (on CBF) can adversely impact tracheo-bronchial MCC. The system can completely fail in HIV infected smokers where TGF-beta signaling is upregulated.

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KSHV induces glutamate secretion and metabotropic glutamate receptor 1 expression: Implications for cell proliferation and targeted therapy

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Although there are numerous diseases associated with increased levels of glutamate release and glutamate receptor expression, their role in virus induced oncogenesis remains unknown. In the present study, we investigated the role of glutamate secretion and glutamate receptor expression in Kaposi's sarcoma-associated herpesvirus (KSHV/HHV-8) infected cells. KSHV is etiologically associated with Kaposi's sarcoma (KS) and primary effusion lymphoma (PEL) in HIV-1 infected patients. Our results demonstrated that elevated glutamate secretion and the metabotropic glutamate receptor 1 (mGluR1) expression play a significant role in KSHV induced cancer cell proliferation. mGluR1 was strongly expressed in KSHV infected primary and latent cells

as well as in the tissue sections from KS and PEL patients. Our data also reveal that the KSHV latency associated nuclear antigen-1 (LANA-1) causes an increase in glutamate secretion by promoting c-Myc activation. In addition, the interaction of another KSHV latent protein, kaposin A, with the neuron-restrictive silencer factor (NRSF/REST) is required for the over-expression of mGluR1. Pharmacological intervention of mGluR1 activity with glutamate release inhibitor riluzole and mGluR1 antagonists significantly inhibited the proliferation of KSHV infected cells. Together, our results demonstrate for the first time that glutamate secretion and mGluR1 expression play crucial roles in the pathological changes associated with KSHV induced malignancies, and provide a firm basis for the development of glutamate based therapeutic drugs for treatment.

EFFECT OF HIV AND COCAINE IN CATHEPSIN B, NOX4 AND SIGMA-1 RECEPTOR EXPRESSION IN POST-MORTEM BRAIN TISSUES

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Human Immunodeficiency Virus (HIV-1) targets CD4+ T lymphocytes,macrophages and microglia. Upon HIV-1 infection, monocytes can pass the blood brain barrier, differentiate into macrophages, serve as viral reservoirs and secrete neuroinflammatory products. One macrophage factor involved in inflammation and neuronal death is cathepsin B, a lysosomal cysteine protease secreted in HIV infected cells (Rodriguez-Franco et al., 2012). Interestingly, cocaine hastens cathepsin B secretion and neurotoxicity in HIV infected macrophages (Zenón-Meléndez et al, 2014); however, its exact mechanism remains unknown. One potential mechanism might be through activation and expression of the sigma-1 receptor, an endoplasmic chaperone that binds to cocaine and controls the expression of the NADPH oxidase 4 (NOX4), a major source of reactive oxygen species. Hypothesis/Objectives: Ïf-1 and NOX4 expression increase concomitantly with cathepsin B expression in post-mortem brain tissues in HIV-infected individuals with cocaine abuse than their non-abusing counterparts. Methods: Anterior basal ganglia tissues were stained fluorescently using a semi-quantitative immunohistochemistry technique and quantified using the Imaris Program

(Bitplane,CT,USA). Point retrieval were analyzed using the Graph Pad Program (CA,USA). Our results demonstrate that cathepsin B; sigma-1 and NOX4 expression increases concomitantly and is statistically significant in HIV infected tissues ($p \leq 0.05$, One-Way ANOVA, Dunn post-test) and mild cognitive impairment cocaine abusers as compared to HIV(-) non-cocaine tissues.

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Morphine disrupts gut homeostasis and induces distinct signatures of gut microbiome and metabolome partially through the TLR2 pathway

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Opioids such as morphine have many beneficial properties as analgesics, however, opioids may induce multiple adverse gastrointestinal (GI) symptoms. We have recently demonstrated that morphine treatment results in significant disruption in gut barrier function leading to increased translocation of gut commensal bacteria. However, it is unclear how opioids modulate the gut homeostasis. By using a mouse model of slow release morphine treatment, we studied effects of morphine treatment on gut microbiome, metabolome, and their interaction with host immune system. We characterized phylogenetic profiles of gut microbes, and found a significant shift in the colonic microbiome following morphine treatment when compared to placebo. Morphine treatment caused dramatic changes in fecal metabolome. Through LC-MS based metabolomics profiling analysis, we identified fatty acid and bile acid metabolism were greatly affected by morphine treatment, implicating the functional changes of gut microbiota. In a longitudinal study, we found naltrexone, an opioid receptor antagonist, reverses the effect of morphine on bile acids, indicating morphine induced changes are opioid receptor dependent. Furthermore, we confirmed that TLR2KO, but not TLR4KO, mouse can alleviate changes of metabolome at day 3 post morphine treatment

compared to wild type mouse, indicating opioid-receptor dependent changes are partially through TLR2 pathway. Our study shed light on effects of morphine on the microbiome-metabolome-host axis, and its role in the gut homeostasis.

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IFN-lambda Inhibits Drug-Resistant HIV-1 Infection of Macrophages

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We previous showed that interferon lambda (IFN-lambda) has the ability to inhibit HIV infection of blood monocyte-derived macrophages (MDM). We have now investigated the effects of IFN-lambda on antiretroviral-drug-resistant HIV infection of MDM. When added to macrophage cultures, IFN-lambda suppressed not only laboratory-adapted HIV Bal strain but also the drug-resistant HIV strains, including AZT-resistant virus (A012) and RT inhibitor-resistant virus (TC49). Moreover, IFN-lambda had synergistic effect on the anti-HIV activity of antiretrovirals (AZT, Efavirenz, Indinavir and Emfuviride) in infected macrophages. Investigation of the mechanism(s) of the IFN-lambda action on HIV showed that IFN-lambda treatment of macrophages induced the expression of tetherin, a cellular restriction factor that blocks HIV release from infected cells. IFN-lambda-treated cells expressed higher level of myxovirus resistance 2 (Mx2), the newly identified HIV post-entry inhibitor that can abolish capsid-dependent nuclear import of subviral complexes, than untreated cells. These observations provide additional evidence to support the potential use of IFN-lambda as a therapeutic and immunomodulatory agent for the treatment of HIV infection.

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Brain-specific glutaminase C overexpression induces neuroinflammation and learning impairment in mice

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Excess excitatory neurotransmitter glutamate is known to trigger a unique form of neurotoxic response that is suspected in the cognitive dysfunction of many neurological diseases, including HIV-1 associated neurocognitive disorders (HAND). Our previous studies revealed upregulation of mitochondrial enzyme glutaminase C (GAC) in the postmortem brain tissues of HAND patients by protein analysis. Because GAC converts glutamine into glutamate, we ration that GAC upregulation may induce excess glutamate that causes the dysfunction and subsequent neuronal damage in HAND. Using a brain-specific GAC transgenic mouse model (overexpression confined in brain), we found that GFAP and Iba1, markers of astrocytes and microglia, were significantly increased in GAC transgenic brains compared with littermate controls, suggesting that elevated GAC induces a neuroinflammatory response. Since the inflammation is pronounced in hippocampus, we further studied the functional impact of GAC overexpression on the learning and memory of the mice through Morris Water Maze test. GAC transgenic mice exhibited longer escape latency during the training and spent less time in the target quadrant during the probe test, indicating an impairment of learning and memory. Together, these data suggest that brain-specific GAC overexpression induces prolonged neuroinflammation and learning impairment in mice.

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Cortical Dysfunction in HIV-1 Transgenic Rats: Effects of Cocaine Self-Administration

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The medial prefrontal cortex (mPFC) is dysregulated in HIV+ humans and by toxic HIV-1 proteins, as well as with chronic exposure to cocaine. Evaluations of mPFC

neuropathogenesis associated with HIV/AIDS, and the co-morbidity imposed by cocaine abuse have been hampered by the historical lack of assessments using animal models of these conditions. Here we reveal the dysregulation that occurs in mPFC pyramidal neurons from HIV-1 transgenic (Tg) F344 rats, with and without self-administration of cocaine. We used adult male Tg and non-Tg rats performing an operant cocaine self-administration (COC-SA) task for 14 days, paired saline-yoked controls (SAL-Yoked), and experimentally naive Tg and non-Tg rats. As cortical hyper-excitability emerges following protracted withdrawal from cocaine exposure, at least 14 cocaine-free days occurred before forebrain slices were harvested for whole-cell patch-clamp recordings. We determined that (i) both genotypes acquired and maintained COC-SA, (ii) withdrawal from COC-SA enhanced excitability in both genotypes, (iii) SAL-Yoked Tg rats exhibited greater excitability than SAL-Yoked non-Tg rats, (iv) surgical instrumentation did not alter the electrophysiological profile of pyramidal neurons in Tg rats, and (v) L-type Ca2+ channels was associated with the increased neuronal excitability. These findings provide the first assessment of mPFC pathophysiology in a rodent model of HIV with cocaine, reveal that the neuropathogenesis is greater in the co-morbid state, and show that L-type Ca2+ channels are involved.

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Exercise maintains blood-brain barrier integrity during early stages of brain metastasis formation

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Tumor cell extravasation into the brain requires passage through the blood-brain barrier (BBB), which is a highly protected microvascular environment fortified with tight junction (TJ) proteins. Tumor cells adhering to the brain endothelium can produce oxidative damage, leading to the activation of redox-sensitive small GTPases, which can disrupt the BBB by targeting TJ proteins for degradation. There is evidence that exercise can alter the oxidation status of the brain microvasculature and protect against tumor cell extravasation and metastasis formation. Mature male mice were housed singly in cages equipped with a running wheel (locked in sedentary) for five weeks. The average

voluntarily running distance was 9.0 ± 2.0 km/day. In order to selectively study the microvascular environment of the BBB, 1x106 D122 cells (murine Lewis lung carcinoma) were infused directly into brain microvasculature through the internal carotid artery. In vivo measurements showed no significant difference between sedentary and exercised mice; however post hoc IHC analysis revealed fewer tumor cells had extravasated into the brain. There was a significant negative correlation between oxidative stress and running distance. Furthermore, exercise decreased redox-sensitive GTPase activity, and expression of tight junction protein, occludin, was maintained in microvessels from exercised mice but not within the sedentary mice. These data indicate that voluntary exercise can influence the microenvironment of the BBB during metastatic progression in the brain.

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Selenium supplementation attenuates early events involved in tumor cell extravasation into the brain by modulating adhesive properties of the brain vascular endothelium

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Dietary agents can influence tumor progression by modulating the first steps towards extravasation, namely tumor cell adhesion and transendothelial migration. We explored whether specific selenoglycoproteins can suppress the interactions of tumor cells with the brain microvascular endothelium and hereby impair tumor cell homing in the brain. Mice were fed diets enriched with yeast-derived selenoglycoproteins (SGP40, SGP65; 1 ppm Se), glycoprotein fractions (GP40, GP65; 0.2-0.3 ppm Se) or a control diet (0.23 mg/kg Se) for 12 weeks. Then, $1.0x10^6$ GFP-Luc-D122 cells (Lewis lung carcinoma) were infused into the brain microvasculature. Mice were euthanized after 48 hours (short-term) or 3 weeks (long-term). In the long-term cohort the development of brain metastatic nodules was monitored using the IVIS Xenogen Bioluminescence Imager. Immunohistochemistry was employed to evaluate adhesion and transcapillary migration of tumor cells. Isolated brain microvessels were used for gene and protein analyses.

Administration of tumor cells usually resulted in a significant upregulation of genes coding adhesion molecules. In the short-term study fraction SGP65 significantly decreased VCAM-1 and ALCAM mRNA levels. Our long-term study revealed markedly decreased expression of ICAM-1 in mice fed SGP65 enriched diets. We observed that specific Se-containing compounds have the potential to attenuate tumor cell adhesion to brain endothelium by decreasing expression of selected adhesion molecules in vivo. Conclusive findings will be informed by the results from the ongoing animal studies.

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MICROVESICLES MEDIATE GLUTAMINASE RELEASE IN HIV-1-INFECTED MACROPHAGES: IMPLICATION FOR HIV-1-ASSOCIATED NEUROCOGNITIVE DISORDERS (HAND)

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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). Our previous studies demonstrated that glutaminase (Gls), a mitochondrial enzyme, is upregulated and released extracellularly, contributing to excess glutamate and neurotoxicity in HAND. However, the mechanism of extracellular release of Gls remains unclear. Microvesicle (MV), a newly identified subcellular particle able to transport cellular contents out of cells, may play a role in Gls release and initiate a host of neurological symptoms. We hypothesized that the release of Gls-containing MVs is a critical pathogenic event in HAND. We demonstrated that HIV-1-infected human primary microglia and monocyte-derived macrophages had significantly higher levels of extracellular MVs under electron microscopy, compared with uninfected controls. Furthermore, we used reverse phase high-performance liquid chromatography and found excess glutamate generation by MVs. Notably, blocking Gls activity with Gls inhibitors in MVs dramatically reduced the glutamate levels, suggesting that MVs released from HIV-1-infected microglia and macrophages are able to produce neurotoxic levels of glutamate through Gls. Consistent with the glutamate levels, MVs-induced neurotoxicity was rescued by Gls inhibitors. To conclude, MVs pathway is a

potential mechanism for Gls release and excess glutamate generation in HAND. Understanding the pathway could help to identify novel therapeutic targets for neuronal protection in HAND.

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Activation of microglia NLRP3 inflammasome underlies methamphetamine-induced neuroinflammation

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Neuroinflammatory responses mediated by microglia over-activation contribute to the neurotoxic effect of methamphetamine (Meth) abuse. IL-1β, a principal proinflammatory cytokine released by activated microglia, was found to be up-regulated after Meth application. The activation of IL-1β is tightly controlled either by Toll-like receptors (TLRs) or Nod-like receptors (NLRs), or both. In microglia, NLRP3 is the predominant NLR against various irritant stimuli and is required for the formation of inflammasome which processes IL-1β into its mature form. It has been shown that exposure of peripheral immune cells to Meth resulted in Ca2+ mobilization, mitochondrial oxidative damage and lysosome alkalization leading to inflammasome activation. We hypothesize that Meth triggers proinflammatory signaling cascade in microglia through activation of NRLP3 inflammasome. To test this hypothesis, we studied Meth-induced inflammasome activation in rat microglia cultures. Treatment of cultured rat cortical microglia with pharmacological concentrations of Meth after LPS priming induced IL-1β and caspase-1 maturation and aggregation of inflammasome adaptor protein ASC demonstrated by significant and dose-dependent increase of cleaved IL-1β and caspase-1 in addition to oligomerization of ASC. All of which suggest an activation of inflammasome. These results demonstrated that NRLP3 inflammasome activate as downstream effect of Meth application. To our knowledge, this is for the first time to show the interplay between inflammasome activation and Meth-associated neuroinflammation.

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Delta -9-Tetrahydrocannabinol-mediates Alterations in Gene Expression in Lymphocytes

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Marijuana has been used for many years for the treatment of nausea and vomiting associated with cancer chemotherapy. Because of its psychotropic effects, it is also a drug of abuse. However, several studies have indicated that it may lead to dysregulation of the immune response δ9-tetrahydrocannabinol (THC) is the main bioactive component in marijuana and its activity is primarily mediated by cannabinoid receptors in immune cells. In this project, we used RNA-seq to quantify the transcriptomes and transcript variants that are differentially regulated by THC in the antigen-activated lymphocytes as well as CD4+ T cells. We have found that the expression of many transcripts were altered by THC in both total lymph node cells and T cell subpopulation. Furthermore, the abundance of many miRNA precursors is dramatically altered in THC treated mice. In addition, THC treatment also causes alternative promoter usage and splicing. The functions of those altered transcripts are mainly related to immune response and cell proliferation.

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MiR-21 in extracellular vesicles leads to neurotoxicity via TLR7 signaling in SIV neurological disease

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HIV associated neurocognitive disorder (HAND) are neurological disorders caused due to the entry of HIV infection in the brain. HIV-1 does not directly infect central or peripheral neurons, however, virus-infected cells of the monocyte/macrophage lineage

maintain a low-level HIV infection in the CNS. "Indirect effects" of macrophage activation--such as dysregulation of cytokines and chemokines, free-radical (oxidative stress) injury, and secretion of soluble factors that are potently neurotoxic--have been implicated as effectors of nervous system injury in HIV. We report that small extracellular vesicles (EVs) released from macrophages can enhance neurotoxicity. Using a nonhuman primate model of HAND, simian immunodeficiency virus encephalitis (SIVE), we find that EVs isolated from SIVE brains contain certain small regulatory RNAs, microRNAs, such as miR-21 that can serve as ligands to the key immune regulatory receptors, toll-like receptors and can elicit neurotoxicity. We provide in vitro evidence for such an effect. Thus, our study provides insights into other potential neurotoxic mechanisms by which HIV infection in the brain could harm neuronal health.

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TNFα induces pain-like behavior and increases spinal p-CREB and p-CEBPβ in rats

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TNFα is one of cytokines that induce inflammation in many diseases. We and others show that TNFα is involved in inflammatory and neuropathic pain. However, the exact downstream pathway of TNFα is still elusive. Evidence shows that transcriptional factors cAMP response element-binding protein (CREB) and CCAAT/enhancer binding protein (C/EBP) beta play a role in the neuroimmuno activity. Here, we tested if TNFα causes pain-like behavior, and changes phospho-CREB and CEBPβ in vitro and in vivo. Male Sprague-Dawley rats were used, and recombinant TNFα was injected intrathecally 4 times twice daily. Mechanical threshold was measured using Von Frey testing. For in vitro experiment, rat neuronal neuroblast cell line B35 was used. Cells were treated with rTNFα or vehicle for 3 hrs. Rat spinal cord tissues or B35 cells were used for western blot. In in vitro studies, rTNFα application to B35 cells increased the expression of pCREB and pCEBPβ compared to control. Blockage of TNFα reduced pCREB and pCEBPβ. Similarly, in in vivo studies, rTNFα intrathecally injected into rats lowered

mechanical threshold, and increased pCREB and pCEBPβ. Inhibition of TNFα reversed the lowered mechanical threshold and suppressed pCREB and pCEBPβ. Our study suggest that pCREB and pCEBPβ may be the downstream factors of TNFα.

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Methanandamide bound to Magnetic Electric Nanoparticle did not compromise resistance and permeability of the Blood Brain Barrier

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Methanandamide (mAEA), a synthetic stable analog of the Endocannabinoid Anandamide (AEA), has been demonstrated to improve motor function and down regulates the production of inflammatory mediators in microglial cells. In HIV infected drug users, significant impairments in neurocognition, increased neurodegeneration and behavioral problems are evident. Therefore, an effective delivery of sufficient mAEA across the blood brain barrier (BBB) to minimize the stimulated abnormal behavior by activating CB1 receptors is needed to control or block cannabinoid-induced behavioral effects in HIV infected drug users. Herein we explored transmigration of a nontoxic concentration of mAEA across blood brain barrier (BBB) using magnetic-electric nanoparticle (MENPs) of BaTiO3CoFe2O4(~20 nm) as a nanocarrier. Results of the studies demonstrated that an optimized nontoxic mAEA concentration was 5 µM-which exhibited beneficial effects on the synaptic plasticity genes. In-vitro BBB studies showed that developed nanoformulation did not compromise the resistance and the permeability of BBB, proven using estimating TEER and paracellular transport. In summary, our designed novel mAEA-MENPs nanoformulation has potentials to deliver mAEA across BBB to treat neurological impairments in cannabinoid using and/or HIV infected patients.

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Neural correlates of cognitive deficits in HIV infection

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Structural MRI and neuropsychological testing (Cambridge Neuropsychological Test Automated Battery (CANTAB)) were acquired in 36 individuals with HIV infection (HIV, 49.5 years) and 33 controls (Ctrl, 44.3 years). Each CANTAB subtest score was standardized on controls' performance and 3 theoretically driven composite scores computed: Attention/Working Memory (ATWM: Intra/Extradimensional Set Shifting, Stockings of Cambridge, Spatial Working Memory), Visual Recognition Memory (VRM: Spatial Recognition Memory, Pattern Recognition Memory), and Decision Making (DM: Cambridge Gambling Test, Affective Go/No Go). HIV scored significantly lower than Ctrl on all 3 CANTAB composite scores: ATWM p=.005, VRM p=.003, DM p = .001. MRI data were quantified for 29 bilateral cortical and subcortical regions. Two regions showed smaller volumes in HIV than Ctrl: lateral frontal cortex (p=.03) and precentral gyrus (p=.005). In the HIV group, ATWM performance correlated with the volume of the temporal lobe (r=.56, p=.0004); VRM performance correlated with volumes of the precuneus (r=.37, p=.03), parietal (r=.36, p=.03), and temporal lobes (r=.40, p=.02)(together explaining 23% of the variance in performance); and DM performance correlated with volumes of the lateral (r=.49, p=.002) and medial (r=.35, p=.04) frontal cortices, and precentral gyrus (r=.40, p=.02)(together explaining 25% of the variance in performance). These results support a relation between decision-making and frontal cortices and visually-based episodic memory tasks and a temporo-parietal network.

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Effects of 3-n-butylphthalide (NBP) on the expression of Bax, Caspase-8 and TH in the substantia nigra of PD rats

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Parkinson's disease (PD) is one of the most common neurodegenerative disorders, involving progressive loss of the nigro-striatal dopaminergic neurons. Currently, there are no efficacious drugs to treat or prevent this disease. Studies in animal model suggest that NBP, a chemical constituent in celery oil, may function as an antioxidant to protect dopamine neurons in PD. To understand the mechanisms underlying NBP-associated neuroprotective effects, we studied its effects on the expression levels of Bax, Caspase-8 and tyrosine hydroxylase (TH) in rats with Parkinsonism. A total of 90 male SD rats were randomly divided into three groups with 30 in each group: the Parkinsonism group, the Parkinsonism+NBP group and control group. The animal Parkinsonism was established by unilateral stereotaxic infusion of 6-hydroxydopamine in right substantia nigra. Animals in Parkinsonism+NBP group were given (i.p.) NBP for 4 weeks and the control animals were injected with saline. After sacrifice animals brain slices contained the substantia nigra were used to detect the mRNA and protein levels of Bax, caspase8 and TH by in situ hybridization and immunohistochemistry analyses. Our results revealed that administration of NBP significantly decreased the expression of Bax and caspase8 protein and mRNA and increased the expression of TH mRNA and protein, implying a protective effect of NBP in animals with Parkinsonism. These results suggest that NBP could be a potential neuroprotective agent for the treatment of PD.

Exosomes mediate cell-to-cell transmission of TLR3-induced antiviral activity and the involvement of microRNAs

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Exosomes are a class of cell-released small vesicles that mediate intercellular communication. During HCV infection, the interaction between liver resident macrophages and hepatocytes is important for host anti-viral responses. We here explored the role of macrophage-derived-exosomes in transmitting innate immunity against HCV infection into hepatocytes. We found that upon TLR3 activation, exosomes shed by macrophages were internalized by Huh7 cells, induced the anti-HCV responses

(type I IFNs, ISGs, etc.), and thus drastically inhibited HCV infection in Huh7 cells. Moreover, using an in vitro macrophage-Huh7-co-culture model, we also found exosomes mediated HCV suppression in Huh7 cells after TLR3 activation. The presence of exosome inhibitor compromised the anti-HCV activity by TLR3-activated macrophages. Interestingly, anti-HCV miRNAs, miRNA-29 family, was significantly elevated in macrophage exosomes by TLR3 activation. Furthermore, the inhibition of miRNA-29 partially compromised the anti-HCV activity of TLR3-activated macrophages, indicating the potential involvement of exosomal miRNAs in exosome-transmitted anti-HCV activity. In conclusion, this study proposed an antiviral mechanism of TLR3 activation that involves the intercellular communication between immune cells and hepatic parenchymal cells via exosomes, and exosome-shuttled miRNAs. This discovery not only sheds light on exploiting the therapeutic potential of new drugs against HCV infection, but also identified a novel model for studying vesicle-mediated cell-to-cell communication in other disease settings.

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