SPEAKER ABSTRACTS

Symposium: Reaching for the Neurotoxicity Society

Organized by Italo Mocchetti, PhD, Georgetown University Medical Center, Washington DC

IS METHAMPHETAMINE USE A RISK FOR PARKINSON'S DISEASE?. Moratalla, R, Ph.D. [1], Ares-Santos, S, Ph.D. [1], Granado, N, Ph.D. [2]; [1]Instituto Cajal, CSIC, Consejo Superior de Investigaciones Científicas, Madrid, 28002[2]CIBERNED, , ISCIII, Madrid, 28002.

Methamphetamine (Meth) is an illicit drug abused by 14-50 million people worldwide. Besides causing addiction, methamphetamine also damages the dopaminergic system in the brain of primates and rodents. In humans, methamphetamine decreases DAT binding sites, shown by PET in drug addicts three years after withdrawal, raising the concern about if methamphetamine can cause Parkinson's disease. In experimental animals it has been clear for some time that Meth causes persistent loss of dopamine fibers, but it is not known if Meth kills dopamine cell bodies. Our study addresses a long-standing question in the field: whether Meth destroys not just the fibers, but also the cell bodies in the substantia nigra, causing permanent damage. To address this question, we studied the effect of Meth on dopaminergic neurotoxicity, by evaluating the integrity of dopamine fibers and cell bodies at different time points using markers of dopaminergic neurons and amino-cupric-silver staining. Strikingly, Meth induced a loss of dopamine neurons of 20-25% at 7 or 30 days. Thus, this is the first demonstration of irreversible Meth-induced neuronal loss. Meth also caused loss of striatal dopaminergic fibers, peaking 1 day postreatment, followed by a progressive recovery. This neuronal damage had functional consequences: mice exhibited a drastic decrease in movement and motor coordination 1 to 3 days after drug delivery. The neuronal loss and fiber damage caused by Meth use likely confers a persistent vulnerability to subsequent insults, increasing the risk of developing Parkinson's Disease
Supported by Spanish Minist Econ y Compet SAF2013-48532R, CIBERNED, ♯ CB06/05/0055, PNSD and Comun Madrid, S2011/BMD-2336.

NEUROINFLAMMATION: DISSECTING MICROGLIA PHENOTYPES AND CONTRIBUTIONS TO INJURY AND REPAIR. Harry, G.J., Ph.D. [1]; [1]Neurotoxicology Group/National Toxicology Program Laboratory, National Institute of Environmental Health Science, Research Triangle Park, NC 27709.

Neuroinflammation is associated with a variety of states within the brain including injury, disease, neurological disorders, and aging however, how this relates to morphological changes in microglia is not clearly characterized. As the primary cellular source for inflammatory factors, microglia serve surveillance, maintenance, and repair functions and display varied phenotypes, some beneficial while others require active regulatory control. Dissecting the distinct phenotypes of microglia subsets as associated with various functions (e.g., clearance of debris, synaptic remodeling, neuronal protection) is a critical step in characterizing unique responses contributing to functional differences. Using the trimethyltin mouse model of tumor necrosis factor receptor-dependent hippocampal injury, resident microglia morphological heterogeneity was examined across hippocampal sub-regions and found to be associated with a spatial and temporal elevation of pro and anti-inflammatory cytokines, iNos, and complement expression. These patterns were differentially associated with neuronal death and synaptic loss. Exploratory protein micro-characterization of the dentate gyrus, CA3 and CA1 pyramidal cell layers

demonstrated unique profiles suggestive of phenotypic classifications of microglial and their environmental niches. In addition, a role for microglia and interleukin 1 beta were demonstrated to facilitate proliferation and survival of hippocampal neuroprogenitor cells, supporting injury-induced hippocampal neurogenesis within a high inflammatory environment.
Supported by USA National Institutes of Health/1Z01ES101623.

ROLE OF TOLL-LIKE RECEPTORS IN THE BRAIN'S INNATE IMMUNE RESPONSE. Maguire-Zeiss, KA, Ph.D. [1]; [1]Neuroscience, Georgetown University Medical Center, Washington, DC 20057.

Microglia play an important role in development, synaptic plasticity, and the brain's response to pathogens, and as such these cells are important for homeostasis. They are in constant motion surveilling the brain for signals that initiate specific cellular cascades resulting in the release of pro- and anti-inflammatory molecules. Pattern recognition receptors (PRRs) are known to mediate these microglial responses. One class of PRRs, toll-like receptors (TLRs), plays an important role in microglial responses to pathogens with specific molecular structures such as bacterial membrane lipopolysaccharide, dsRNA, viral proteins, and CpG DNA. More recently, TLRs have been implicated in the inflammation associated with neurodegenerative disorders. In these diseases, TLRs are thought to respond to danger/damage-associated molecular patterns such as misfolded and pathogenic proteins. Here we detail complex morphofunctional microglial responses to various pathogens via TLR activation. The pathways presented are relevant to neuroinflammation such as seen in Parkinson's disease and HAND. Specifically, using primary mouse microglia, we show that the Parkinson's disease relevant protein α-synuclein directly activates TLR1/2 inciting the expression of both pro- and anti-inflammatory molecules and PAR-1, an altered morphology, and increased production of miRNAs known to regulate this signaling pathway. Furthermore, brain slices from CX3CR1-GFP/+ mice exposed to oligomeric α-synuclein show an increased microglial response when compared to non-pathogenic monomeric protein.
Supported by PMDF; NIH R01NS083410.

PROBDNF, P75NTR AND HIV-MEDIATED NEUROTOXICITY. Mocchetti, Italo, Ph.D. [1], Tessarollo, L, Ph.D. [2], Bachis, A, Ph.D. [1]; [1]Department of Neuroscience, Georgetown University, Washington, DC 20057[2]MCGP, National Cancer Institute, Frederick, MD 21702.

Human Immunodeficiency Virus-1 (HIV) and its envelope protein gp120 promote axonal retraction and dendritic simplification. However, the mechanisms contributing to these pathological features are still not completely understood. The proneurotrophin brain-derived neurotrophic factor (proBDNF), whose expression is up-regulated by gp120, promotes synaptic simplification through the activation of the p75 neurotrophin receptor (p75NTR), a member of the tumor necrosis factor family. In this study, we have used wild type and gp120 transgenic (gp120tg) mice to investigate whether p75NTR has a role in gp120-mediated neurotoxicity. Old (~10 months) gp120tg, but not adult (~3 months) animals exhibited an increase in proBDNF levels in the hippocampus as well as a decrease in the number of dendritic spines when compared to wild type controls. To test if the reduction in spine density and morphology is caused by the activation of p75NTR, we crossed gp120tg mice with p75NTR null mice to generate gp120tg/ p75+/-mice. We found that deletion of only one copy of the p75NTR gene in gp120tg mice is sufficient to normalize the number of hippocampal spines, strongly suggesting that the

neurotoxic effect of gp120 is mediated by p75NTR. These data indicate that p75NTR antagonists could provide an adjunct therapy against synaptic simplification caused by HIV.
Supported by HHS NS079172, NS074916.

Symposium: Neuroinflammation and the Gut: What is its relevance to HIV infection, neurological pathology, and therapy?

Organized by T. Celeste Napier, PhD, Rush University Medical Center and Peter J. Gaskill, PhD, Drexel University College of Medicine

(Hamid Akbarali, Vice Chair of Pharmacology and Toxicology, and Director, Graduate Education and Postdoctoral Training and Amanda Persons, a newly appointed assistant professor from Rush University) as well as a non-SNIP member (Satya Dandekar, Chair of the Department of Medical Microbiology and Immunology at UC Davi

Symposium: Neurobiology of exercise in drug abuse and neurodegenerative diseases. Chairs: Adam Zajac and Wendy J. Lynch

Michael Jakowec (USC) “Circuit specific neuroplasticity: a novel approach to treat degenerative brain disorders”

Sonata Suk-yu Yau (Hong Kong Polytechnic University) “Tracking the antidepressant effects of physical exercise and its mechanisms”

Jose Vina (Univ. Valencia, Spain) “Physical exercise in the prevention and treatment of Alzheimer’s disease”

Wendy J. Lynch (University of Virginia School of Medicine) “Mechanisms for the Efficacy of Exercise as an Intervention for Drug Addiction”

TRACKING THE ANTIDEPRESSANT EFFECTS OF PHYSICAL EXERCISE AND ITS MECHANISMS. Yau, SY, Ph.D. [1], Li, A, Ph.D. [2], Christie, BR, Ph.D. [3], Xu, AM, Ph.D. [4], So, KF, Ph.D. [6]; [1]Department of Rehabilitation Sciences, Hong Kong Polytechnic University, Hong Kong, 852[2]Guangdong-Hong Kong-Macau Institute of CNS Regeneration, Guangdong Key Laboratory of Brain Function and Diseases, Jinan University, Guang Dong, 510632[3]Division of Medical Sciences, University of Victoria, Victoria, V8P5C2[4]Department of Medicine, University of Hong Kong, Hong Kong, 852[5]State Key Laboratory of Brain and Cognitive Sciences, University of Hong Kong, Hong Kong, 852[6]Department of Ophthalmology, University of Hong Kong, Hong Kong, 852.

Physical exercise is known to be beneficial for alleviating depression; however its underlying mechanisms are still unclear. Our previous animal studies have shown that physical exercise in terms of voluntary wheel running elicits it antidepressant effect via increasing adult neurogenesis and dendritic complexity in the dentate gyrus of the hippocampus. To further study how physical exercise enhances hippocampal plasticity, we targeted an adipocyte-secreted hormone: adiponectin which exhibits anti-inflammatory, insulin-sensitizing, and anti-diabetic properties just like exercise. Adiponectin can pass through the blood–brain barrier and its levels in the hippocampus can be increased by physical exercise.

Administration of recombinant adiponectin into the brain decreases depression-like behaviors and promotes hippocampal neurogenesis, whereas genetic knockout of adiponectin diminishes the neurogenic and antidepressant effects of exercise. Recent studies reported that adiponectin has a rapid antidepressant effect independent to hippocampal neurogenesis. Our latest data have shown a novel role of adiponectin in promoting synaptic plasticity in the hippocampus by modulating N-Methyl-D-aspartate receptor function. These results not only reveal possible mechanisms by which exercise exerts its antidepressant effect, but also suggest a potential therapeutic treatment for depression by up-regulating adiponectin levels.
Supported by The work is supported by Hong Kong Health and Medical Research Fund and by funds of Leading Talents of Guangdong (2013), Program.

MECHANISMS FOR THE EFFICACY OF EXERCISE AS AN INTERVENTION FOR DRUG ADDICTION . Lynch, WJ, Ph.D. [1], Beiter, RM, MS [1], Peterson, AB, Ph.D. [1], Abel, J, Ph.D. [1]; [1]Psychiatry and Neurobehavioral Sciences, University of Virginia, Charlottesville, VA 22901.

Exercise has shown promise as a non-pharmacological prevention and intervention for addiction. In this talk, I will present evidence from preclinical and clinical studies for the efficacy of exercise at different phases of the addiction process focusing on psychostimulant addiction. The neurobiological mechanisms underlying the efficacy of exercise at reducing vulnerability to addiction will also be discussed focusing on dopaminergic and glutamatergic signaling in the reward pathway, signaling pathways critically involved in addiction, as well as long-term epigenetic-regulated changes in this brain region. Evidence suggests that the mechanisms for the efficacy of exercise as a prevention or intervention for addiction vary with the level, type, and timing of exercise exposure, and the stage of addiction. We conclude with future directions for the development of exercise as a treatment for addiction including a tailored approach for addiction stage as well as its use as an adjunct to other strategies.
Supported by NIDA/R01 DA039093.

Marta Skowronska (University of Miami School of Medicine and Jerzy Kukuczka Academy of Physical Education, Katowice Poland) “Methamphetamine and HIV-induced aberrant neurogenesis: protection by exercise” (a shorter talk by a junior investigator)

Local Organizers Symposium

Organized by Professor Marta Kubera, PhD and Professor Wladyslaw Lason, PhD, Institute of Pharmacology, Polish Academy of Sciences, Krakow, Poland

MYELOPEROXIDASE - HALIDE SYSTEM IN INNATE IMMUNITY. A ROLE OF TAURINE.. Marcinkiewicz, J, MD, Ph.D. [1]; [1]Department of Immunology, Jagiellonian University Medical College, Kraków, 31-121.

Taurine, the most abundant free amino-acid in mammalian tissues, reaches particularly high concentrations in the brain, retina, muscles and neutrophils. Taurine has been shown to be tissue-protective in many models of oxidant-induced injury. At a site of inflammation, taurine is a scavenger of hypochlorous acid (HOCl), the major product of the neutrophil myeloperoxidase (MPO)–halide system. HOCl, is not only a key molecule of the host defense against microbes but it is also responsible for the tissue injury, when generated in excess. Importantly, HOCl-oxidative modification (chlorination) of enzymes alters their biological functions. The reaction of HOCl with taurine results in a formation of taurine chloramine (TauCl), the less toxic agent with anti-inflammatory and anti-microbial properties. Therefore, the primary role of taurine in inflammation is protecting self-components of the immune system from HOCl-mediated oxidative damage. This talk will focus on the biological effects of neutralization of HOCl by taurine. Our results indicate that taurine conversion of HOCl to TauCl is associated with amelioration of biological effects of protein chlorination. In conclusion, a number of data indicate that taurine is a pivotal component of the MPO-halide system and modifies its impact on innate and adaptive immune system. However, immunoregulatory activities of endogenous taurine and TauCl are masked in vivo due to the redundancy of the immune system.

MICROGLIA PLASTICITY - MOLECULAR CHARACTERISTICS OF DIFFERENT PHENOTYPES IN NEUROPATHOLOGIES. Kaminska, B, Ph.D. [1], Gieryng, A, Ph.D. [1], Rayan, WD, MS [1], Kocyk, M, MS [1]; [1]Laboratory of Molecular Neurobiology, Neurobiology Center, Nencki Institute, Warsaw, 02-093.

Microglia and peripheral macrophages accumulate and respond to signals originating in the injured, infected or inflammed brain. Gliomas attract microglia and macrophages and re-programm them into immunosuppressive cells promoting tumor progression. The molecular signature of myeloid cells in the inflammed brain and specific roles of different populations are still unclear. Lack of robust markers differentiating microglia from macrophages makes it less feasible. Our studies of putative microglial and macrophage genes in cultured microglia exposed to glioma or lipopolysaccharide (LPS) show switching a phenotype and polarization rather than a fixed profile which indicates the functional plasticity of microglia. Using transcriptomic and functional assays we found activation of non-overlapping transcriptional, signaling and metabolic pathways in primary microglia cultures undergoing inflammatory or glioma associated activation. Glioma-secreted factors enhanced "healing" properties of microglia. Gene expression studies in sorted microglia/macrophages (CD11b+ cells) from human high grade tumors and rodent experimental gliomas indicated their re-programming into pro-tumorigenic M2-like cells in glioma microenvironment. Sorting of myeloid populations from ischemic brains (a transient middle cerebral artery occlusion model, MCAo) revealed a prevalence of inflammatory M1 microglia 1 day after MCAo and accumulation of M2-macrophages 3-7 daysafter MCAo. Our results show functional plasticity of microglia and its distinctive functions in neuroinflammation and repair. Supported by Supported by a grant 2012/04/A/NZ3/00630 from the National Science Center, Poland.

IMPORTANCE OF MICROGLIAL ACTIVATION IN NEUROPATHIC PAIN. Mika, J, Ph.D. [1]; [1]Department of Pain Pharmacology, Institute of Pharmacology Polish Academy of Sciences, Krakow, 31-343.

Neuropathic pain may now be considered as neuro-immune disorder, since it is known that the spinal activation of glia results in the release of both pro- and anti-nociceptive cytokines. The microglial production of immune factors is believed to play an important role also in opioids effectiveness. Paradoxaly opioids can induced both analgesia and algesia. It has recently been hypothesized that the imbalance between the pro- and anti-nociceptive activity of peptides generated from opioid prohormones is leading to the pain development. Our results show that minocycline diminished spinal prodynorphin level that were previously elevated following nerve injury. Besides, the minocycline enhanced the analgesic effects of low-dose dynorphin and prevented the development of flaccid paralysis following high-dose, suggesting neuroprotective effect. We have indicated an important role of some pronociceptive cytokines in the dynorphin-neurotoxic. Interestingly, primary microglial cell culture studies confirmed the presence of MOR&KOR and provide evidence for the lack of DOR. Interestingly, the analgesic effect of DOR ligands (DPDPE,deltorphine II) under neuropathy is not diminished in contrast to MOR/KOR ligands (morphine,DAMGO,U50.488H,SNC80). Summing up, the DOR analgesia is different from analgesia induced by MOR/DOR because it does not dependent on injury-induced microglial activation. Therefore, DOR agonists appear to be the best candidates for new drugs to treat neuropathic pain. Our study provide evidence for some new important mechanisms underlying the development of neuropathy

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PHARMACOLOGICAL ACTIVATION OF MITOCHONDRIAL ALDEHYDE DEHYDROGENASE (ALDH2) IN ANIMAL MODELS OF DEPRESSION AND NEURODEGENERATION – SUMMARY OF THE BEHAVIORAL, BIOCHEMICAL AND PROTEOMIC DATA . Olszanecki, R, MD, Ph.D. [1], Stachowicz, A, Ph.D. [1], Głombik , K, Ph.D. [2], Basta-Kaim, A, Ph.D. [2], Suski, M, Ph.D. [1], Lasoń, W, Ph.D. [2], Kubera, M, Ph.D. [2], Adamek, D, Ph.D. [3], Korbut, R, MD, Ph.D. [1]; [1]Chair of Pharmacology, Jagiellonian University Medical College (JUMC), Kraków, 31-531[2]Department of Experimental Neuroendocrinology, Institute of Pharmacology, Polish Academy of Science (IP PAS), Kraków, 31-343[3]Chair of Pathomorphology, Jagiellonian University Medical College, Kraków, 31-531.

Recently, it has been shown that mitochondrial dysfunction and oxidative stress play an important roles in the pathogenesis of mood disorders and neurodegeneration. Mitochondrial aldehyde dehydrogenase (ALDH2), an enzyme responsible for the detoxification of reactive aldehydes – the end products of lipid peroxidation, is considered to exert protective role in mitochondria. We tested Alda-1, a small-molecule activator of ALDH2 in animal model of depression (the prenatally stressed rats) and mild neurodegeneration (apolipoprotein E knockout mice, apoE-/-). Administration of Alda-1 resulted in attenuation of depressive- and anxiety-like behaviors in prenatally stressed rats. Such effects were associated with decrease of tumor necrosis factor (TNF)-α, increase of peroxisome proliferator-activated receptor α coactivator (PGC)-1α and changes in expression of several proteins related to mitochondrial function in the frontal cortex and hippocampus of the prenatally stressed rats, as well as with normalization of peripheral immune parameters. Prolonged treatment with Alda-1 led also to the beneficial changes in expression of factors related to neuroplasticity, memory formation and

mitochondrial function in the frontal cortex and hippocampus of apoE-/-. In conclusion, ALDH2 activation by Alda-1 attenuated of behavioral and immunological abnormalities in the prenatally stressed rats, among others reduced level of inflammatory markers in brain. Moreover, the pattern of mitoproteome changes in both, prenatally stressed rats and apoE KO mice suggested mitoprotective effect of Alda-1.
Supported by JUMC (K/ZDS/003802), NCN (2011/01/N/NZ4/01145, 2011/01/N/NZ4/01142, 2011/01/N/NZ2/00089), IP PAS.

REGULATION OF GR-DEPENDENT GENES IN THE BRAIN IN RESPONSE TO PSYCHOTROPIC DRUGS.
Korostynski, M, Ph.D. [1], Piechota, M, Ph.D. [1], Golda, S, Ph.D. [1], Ficek, J, BS [1], Zygmunt, M, MS [1], Przewlocki, R, Ph.D. [1]; [1]Department of Molecular Neuropharmacology, Institute of Pharmacology PAS, Krakow, 31-537.

Complex etiology and heterogeneity of mental disorders are associated with moderate effectiveness of available drugs. The identification of common and specific neurobiological factors of psychotropic drugs is critical to understanding therapeutic mechanisms. Psychoactive drugs stimulate expression of genes and translation of new proteins that are required for treatment related adaptations in the brain systems controlling drive and motivation. We used whole-genome microarray and NGS (next-generation sequencing) profiling to evaluate time-course of transcriptome alterations following acute drug administration. The results indicated the major drug-regulated expression pattern in the striatum. We found glucocorticoid receptor (GR) as the main factor regulating large network of drug-inducible transcripts. Functional links that connect expression of this transcriptional network to the control of brain metabolism (Sul2a1), immunological response (Tsc22d3) and cell migration (Plat) were found. Furthermore, we identified in the brain specific GR-dependent gene isoforms responsive to drug treatment. Additional analyses suggested glial cells to be the locus of these changes. Our results indicate that psychotropic drugs induce in the brain genomic program targeting GR-dependent genes. Several lines of evidence indicate important role of inducible gene expression in glia cells in formation of drug treatment-related neuroplastic alterations.
Supported by NCN grant 2011/03/D/NZ3/01686.

CHANGES IN DNA METHYLATION IN RAT BRAIN STRUCTURES AFTER COCAINE SELF-ADMINISTRATION.
Sadakierska-Chudy, A. [1], Przegaliński, E. [1], Filip, M. [1]; [1]Laboratory of Drug Addiction Pharmacology, Institute of Pharmacology PAS, Krakow, 31-343.

Cocaine is a psychostimulant drug that repeated treatment induces long-lasting changes in brain neuroplasticity. Hippocampus is one of the most important structures of limbic system involved in addictive process. Many studies indicate that cocaine induces dynamic changes in DNA methylation governing synaptic plasticity, learning and memory formation. The purpose of this study was to assess promoter methylation status in hippocampal genes during early (3.day) and late (10. day) extinction training following cocaine self-administration. The intravenous cocaine self-administration (access for 2h/day; 0.5 mg/kg/infusion) and `yoked` procedure were used in rats to obtain brain tissue for meDIP-chip analysis. Gene ontology (GO) analysis revealed that methylated genes are involved in signal transduction, transcription, mRNA splicing and protein trafficking. Moreover, with pathway analysis we found changes in genes implicated in Wnt and MAPK signaling pathway as well as regulation of

cytoskeleton, SNARE interactions in vesicular transport, neuroactive ligand-receptor interaction, spliceosome and oxidative phosphorylation. Our findings suggest that extinction training followed by cocaine self-administration influences methylation pattern at the promoter of genes engaged in neuronal function and synaptic plasticity thus may have an impact on the hippocampal learning and memory tracks.
Supported by Supported by the statutory fund of the Institute of Pharmacology and the National Science Centre grant no. UMO-2012/06/A/NZ3/000.

Symposium: Astrocyte-neuron interaction: metabolism, ion homeostasis and impulse propagation.

Organizers: Jan Albrecht, Magdalena Zielińska (Warsaw, Poland)

CONTROL OF CA2+ SIGNALING, FUNCTIONS AND SURVIVAL, BY THE MITOCHONDRIAL NA+/CA2+ EXCHANGER NCLX IN GLIA AND NEURONS.. Sekler, Israel 1; 1Dept. of Physiology and Cell biology, Faculty of health sciences Ben-Gurion University , Beer-Sheva, 94875.

Mitochondria are the energy hub of the cell, but are also central to Ca2+ signaling. Mitochondrial Ca2+ uptake driven by the membrane potential enters the mitochondria and is then pumped out through a mitochondrial Na+/Ca2+ exchanger NCLX . This mitochondrial ca2+ shuttling couple Ca2+ signaling to metabolic activity and also control the local Ca2+ concentration at ER and plasma membrane domain At the first part of my talk I will describe the role of the mitochondrial Na+/Ca2+ exchanger, NCLX in astrocytes We found that NCLX is responsible for astrocytic mitochondrial Ca2+ extrusion. Inhibition of NCLX function modulated cytosolic Ca2+ dynamics in astrocytes and had a strong effect on Ca2+ influx via store-operated entry. In contrast, ER Ca2+ release triggered only modest mitochondrial Ca2+ transients, indicating that the functional cross talk between the plasma membrane and mitochondrial domains is particularly strong in astrocytes. Finally molecularly controlling NCLX shaped Ca2+-dependent processes in astrocytes such as exocytotic glutamate release, wound closure, and proliferation. In the final part of my talk I will describe a novel PKA dependent regulatory site of NCLX that unregulated NCLX activity following mitochondrial depolarization encountered in neurodegenerative models.

Supported by ISF DIP.

SODIUM HOMEOTASIS AND SIGNALLING IN THE CNS. Rose, CR, Ph.D. [1]; [1]Institute of Neurobiology, Heinrich Heine University Duesseldorf, Duesseldorf, 40225.

Animal cells maintain a steep inward electrochemical gradient for sodium. This gradient energizes ion regulation and provides the basis for action potentials and excitatory postsynaptic currents in neurons. It also drives reuptake of transmitters, a task mainly performed by astrocytes. Because of its vital importance, intracellular sodium of both neurons and astrocytes was thought to be kept at a stable level. Contrary to this, our recent work, combining dynamic imaging of intracellular sodium with the fluorescent indicator SBFI with whole-cell patch-clamp in mouse hippocampus, has uncovered the existence of transient sodium changes with neural activity. We found that active neurons experience significant sodium increases upon excitatory synaptic transmission due to influx of sodium through

glutamate-gated ion channels. Excitatory activity also evoked long-lasting sodium transients in astrocytes, which were mainly due to sodium-dependent glutamate uptake. The kinetic and spatial properties of activity-related sodium transients were fundamentally different from those described for calcium signals. The functional consequences of these sodium transients are just coming into view. Our own work shows that sodium elevations diminish glutamate uptake capacity by astrocytes. Furthermore, sodium increases promote reversed uptake of calcium by the Na+/Ca2+-exchanger, thereby contributing to intracellular calcium transients. Sodium changes might thus serve as signals themselves, influencing and regulating important cellular functions and playing a role in neuron-glia interaction.
Supported by German Research Foundation, Priority Program 1757 "Glial Heterogeneity" (DFG Ro2327/6-1,8-1).

THE SLC38 FAMILY OF GLUTAMINE TRANSPORTERS AND THEIR CONTRIBUTION TO THE GLUTAMATE/GABA-GLUTAMINE CYCLE AND BRAIN PATHOLOGY. Chaudhry, FA, MD, Ph.D. 1, Moen, MN, Ph.D. 1, Andersen, AN, MS 1, Hamdani, EH, Ph.D. 1; 1Department of Molecular Medicine, Institute of Basic Medical Sciences, University of Oslo, Oslo, NE 0372.

According to the glutamate/GABA-glutamine cycle hypothesis glutamate and GABA are released exocytotically to activate their cognate receptors. The signal is terminated by transport of the neurotransmitters to a large extent into astroglial cells for conversion to glutamine which is then shuttled back to the nerve terminals for resynthesis of the neurotransmitters. Although existence of such a cycle is widely accepted, the mechanism and regulation of glutamine transport across astroglial and neuronal membranes remains elusive. We have provided compelling evidence that the system N transporters SN1 (Slc38a3) and SN2 (Slc38a5) reside on astroglial membranes and release glutamine. The homologous system A transporters SAT1 (Slc38a1) and SAT2 (Slc38a2) are localized on the cell membranes of GABAergic and glutamatergic neurons, respectively, and mediate electrogenic and unidirectional transport that allows for accumulation of glutamine in neurons for resynthesis of GABA and glutamate. We have now identified novel proteins and ions which interact with these transporters and regulate their activity. This perturbs astroglial-to-neuronal shuttling of glutamine, impairs normal brain functions and contributes to development of several brain diseases.

Supported by The Polish-Norwegian Research Program (Project Contract No Pol-Nor/196190/23/2013)

UPREGULATION OF THE Y+LAT2 TRANSPORTER IS RESPONSIBLE FOR COUPLING OF INCREASED ARGININE UPTAKE TO NITROSATIVE STRESS IN AMMONIA-OVEREXPOSED ASTROCYTES. Zielińska, M., Ph.D. [1]; [1]Department of Neurotoxicology, Mossakowski Medical Research Centre, Polish Academy of Sciences, Warsaw, 02-106.

Increased activity of the nitric oxide (NO)-cyclic GMP pathway in the brain is considered the major immediate causes of oxidative/nitrosative stress associated with ammonia neurotoxicity. Increased uptake of the NO precursor, arginine (Arg), contributes to increase NO synthesis and oxidative/ nitrosative stress in ammonia-overexposed CNS tissues. In the CNS, Arg enters the cells mainly via transport systems y+ and y+L. Involvement of y+–mediated Arg in the ammonia-induced NO synthesis has been demonstrated in neurons. By contrast, the identity of Arg transporter(s) involved in ammonia-

induced Arg uptake and its subsequent metabolism to NO in astrocytes has long remained unclear. y+LAT2 is a member of the heteromeric y+L system transporters that catalyze both unilateral Arg transport and its exchange for glutamine. In hyperammonemia in vivo, increased extracellular accumulation Gln in the brain was found associated with reduction of Arg conversion to NO and cGMP synthesis triggered by y+LAT2-mediated Gln influx/Arg efflux. Next, in cultured rat cortical astrocytes, ammonia-induced increase of Arg uptake was found coupled to an increase of the y+LAT2, but not of y+ expression and activity, and further to increase NO production, iNOS expression, and deleterious 3-nitro-tyrosylation of astrocytic proteins, as all the three above effects of ammonia were abrogated by pretreatment of astrocytes with either siRNA complementary to y+LAT2 mRNA or y+LAT2 antibody. The above described events were absent in ammonia-overexposed cultured cerebellar neurons.

Joint SIF/SNIP Symposium

Organized by Marco Cosentino, MD, PhD (SIF) and Sanjay Maggirwar, PhD (SNIP)

DOPAMINE AT THE CROSSROAD OF NEURAL, IMMUNE AND INFECTIOUS DISEASE. Cosentino, M, MD, Ph.D. [1]; [1]Center for Research in Medical Pharmacology, University of Insubria, Varese, 21100.

Dopamine (DA) is a neurotransmitter involved in crucial central nervous system (CNS) functions including motivation, cognition, movement and reward. DA is however increasingly recognized for its role in the neuroimmune network, contributing to the CNS-immune system interplay and in the communication among immune cells. DA affects possibly all human immune cells, including T and B cells, dendritic cells, monocytes/macrophages, microglia, neutrophils and NK cells. DA is also produced by immune cells, and may act on immune cells themselves as well as on neighbouring cells. Results from animal models and clinical studies support the involvement of dopaminergic pathways in immune cells in several diseases, including: multiple sclerosis, Parkinson's disease, cancer, HIV infection. Emerging evidence indicates the occurrence of dopaminergic immune mechanisms also in metabolic disease and in the regulation of hematopoiesis. DA acts on 5 different dopaminergic receptors grouped into two families: the D1-like and the D2-like. Pharmacological modulation of dopaminergic pathways can be obtained also by use of indirectly acting agents targeting DA synthesis, storage and release, uptake and metabolism. Many directly and indirectly acting dopaminergic drugs are in use for non-immune indications (e.g. cardiovascular, neurologic, neuropsychiatric) with a favourable therapeutic index, representing therefore valuable opportunities for drug repurposing in the neuroimmune network. Supported by Fondazione CARIPLO - Project 2011-0504: Dopaminergic modulation of CD4+ T lymphocytes: relevance for neurodegeneration.

ROLE OF GLUCOCORTICOID INDUCED LEUCINE ZIPPER (GILZ) IN MEDIATING THE ANTI INFLAMMATORY EFFECT OF GLUCOCORTICOIDS. Riccardi, Carlo, MD, Ph.D. [null]; [1]Section od Pharmacology, Department of Medicine, Perugia, 06132.

Glucocorticoids (GC) exert important therapeutic effects in many inflammatory/autoimmune and degenerative diseases. Most of GC effects are receptor (GR) mediated and relate to regulation of gene transcription. Notably, GR modulates neuronal functions and viability through both genomic and non-genomic actions, and importantly its transcriptional regulatory activity tightly correlates with GC effects. Moreover, there is increasing evidence pointing to involvement of GC-GR in neurodegenerative and

cognitive disorders and GC are used in therapy of neurodegenerative diseases. The knowledge of molecular mechanisms of GC is important to define appropriate treatment schedules and identify new potential targets for therapy. With the aim to deeply analyze the mechanisms of GC and discover new putative molecular targets, we identified GC-induced GILZ (Glucocorticoid-Induced Leucine Zipper), a protein rapidly induced by GC. Our results indicate GILZ as a mediator of GC effects that modulates NF-kB, Ras/MAPK pathway, and SMAD system activity. GILZ regulates T cell activation, apoptosis, differentiation, cytokine production and inflammation. Analysis of T lymphocytes subpopulations shows that GILZ, similar to GC, influences Th1/Th2 ratio and stimulates T regulatory (Treg) cells development. Of note, GILZ is essential for GC-induced expression of Foxp3 and Treg number increase, thus indicating GILZ is an important anti-inflammatory molecule. In conclusion, results indicate GILZ as a mediator of GC activity, a target for new anti-inflammatory drugs and suggest new therapeutic approaches.

SHORT AND LONG TERM EFFECTS OF DELTA-9-TETRAHYDROCANNABINNOL ON CYTOKINES IN ADULT AND ADOLESCENT MICE . Sacerdote, P [1]; [1]Department of Pharmacological and Biomolecular Sciences, University of Milano, Milano, 20129.

Early exposure to Delta-9-Tetrahydrocannabinol (THC) is associated with immediate and long term deleterious effects. In adolescence an elevated incidence of THC abuse is reported. THC is able to modulate immune responses and cytokine production both in the periphery and in the Central Nervous System (CNS). The main objectives of our study was to evaluate in the mouse whether the exposure to THC in adolescence may induce immediate and delayed effects on immunity that might persist in adulthood. As peripheral immune parameters we considered both innate and acquired immunity, measuring T-cells and macrophage cytokine production. Since cytokines are produced also in CNS by microglia and astrocytes, where they have a role in neuroinflammation, we evaluated also cytokines in the hypothalamus and hippocampus. Adolescent mice were treated with THC for ten days, and cytokines measured immediately at the end of treatment and 80 days later in adult age. Our results on peripheral immunity showed that whereas THC switched the cytokine balance towards an anti-inflammatory profile immediately after treatment, in adulthood it induced a long-lasting opposite modulation of immune responses after a washout period, characterized by a shift towards a proinflammatory macrophage and T-helper-1 phenotype. Similarly, also brain cytokines are shifted towards a proinflammatory profile in adult mice exposed to THC in adolescence. In conclusion, it can be stated that adolescence is a vulnerable period for THC-induced modulation of cytokines both in the peripheral immune system and in CNS

Supported by Dipartimento Politiche Antidroga, Presidenza del Consiglio dei Ministri, Italy.

Symposium: Glial cells in neurodegeneration."

Organized by Professor Alexej Verkhratsky from Manchester, UK.

GLIA-ENDOCRINE SYSTEM AND NEUROLOGICAL DISORDERS. Noda, Mami, Ph.D. [1]; [1]Pharmaceutical Sciences, Kyushu University, Fukuoka, 812-8582.

There is a close relationship between the endocrine system and the central nervous system (CNS). Among hormones closely related to the nervous system, thyroid hormones (THs) are critical for the development and function of the CNS; not only for neuronal cells but also for glial development and

differentiation. Any impairment of TH supply to the developing CNS causes severe and irreversible changes in the overall architecture and function of human brain, leading to various neurological dysfunctions. In adult brain, impairment of THs, such as hypothyroidism and hyperthyroidism, affect immune system, potentially increase the risk of cognitive impairment, Alzheimer's disease (AD), and cause psychiatric symptoms such as schizophrenia, bipolar disorder, anxiety and depression. It is known that hypothyroidism impairs synaptic transmission and plasticity, however, its effect on glial cells and cellular mechanisms are not well known. We have observed the effects of THs on glial morphology and function. THs activated microglial migration both in vitro and in vivo. In mouse brain with both hypothyroidism and hyperthyroidism, morphologically activated microglia and astrocytes were observed, which were age- and sex-dependent. It was suggested that endocrine system has fundamental role on glial cells in a complicated manner and therefore affects neuronal circuit in adult brain. Consequently, it may help to understand physiological and/or pathophysiological functions of THs in the CNS and how hypothyroidism and hyperthyroidism may cause neurological and mental disorders.

WHY WHITE MATTER MATTERS: RELATIONSHIP BETWEEN DYSREGULATION OF NEUROTRANSMISSION, MYELIN LOSS AND COGNITIVE DECLINE . Butt, A.M., Ph.D. [1]; [1]Institute for Biomedical and Biomolecular Sciences, University of Portsmouth, Portsmouth, PO1 2DT.

The massive computing power of the brain depends on myelinated fibres that are bundled together into the white matter (WM). Rapid neural communication depends on the life-long production of myelin by oligodendrocytes, which are generated from oligodendrocyte precursor cells (OPCs). Neurotransmitters released by neurons regulate the differentiation of OPCs into oligodendrocytes. Although lacking neuronal synapses, experimental studies on WM support a model of neurotransmitters being released from axons during action potential propagation to regulate myelination, with prominent roles for glutamatergic, purinergic (ATP and adenosine) and GABAergic signalling. Notably, myelination declines in the ageing brain, which is associated with cognitive decline and white matter loss in Alzheimer's disease (AD). Our studies provide evidence that OPCs are disrupted in the ageing brain and that this is accelerated in mouse models of AD. These changes are correlated with a dysregulation of neurotransmitter signalling in ageing WM of the mouse optic nerve, comparable to that described in the GM of human ageing brain and AD. This lead us to propose a vicious cycle of altered neurotransmitter signaling, impaired OPC regenerative potential and myelin loss, which may be important in the loss of WM in AD.

Supported by Supported by the BBSRC.

ASTROCYTES, EXCITATION-ENERGY COUPLING AND VESICLE-BASED SIGNALING . ZOREC, R., Ph.D. [1], Vardjan, N., Ph.D. [2], Kreft, M., Ph.D. [3], Chowdhury , H.H., Ph.D. [1], Horvat, A., MS [1], Stenovec, M., Ph.D. [2], Lasič, E., MS [1], Rituper, B., MD, Ph.D. [1], Jorgačevski, J., Ph.D. [2], Potokar, M., Ph.D. [1], Gabrijel, M., Ph.D. [1]; [1]Univerza v Ljubljani, Medicinska fakulteta, Inst. Pathophysiol. Lab Neuroendo-Mol. Cell Physiol., Ljubljana, SC 1000[2]Celica BIOMEDICAL, Lab Cell Engineering, 1000, 1000[3]Univerza v Ljubljani, Biotehniška fakulteta, Dept. Biology, Ljubljana, 1000.

Astrocytes, a heterogeneous glial cell type, get excited when neurotransmitters bind to their membrane receptors and feed-back by releasing their own signals. This involves vesicles, which store chemicals termed gliotransmitters or more generally gliosignaling molecules. In the former case

chemical messengers get released from astrocytic sites proximal to the synapse, which defines communication to occur in the micro-space of contact between the synapse and the astrocyte. In contrast gliosignaling molecules may also be released into the extracellular space and get transported to locations far away from the active astrocyte. This mode of release resembles the endocrine system. Hence astrocytes are considered to be part of the gliocrine system in the brain, where the glymphatic system mediates the convection of released molecules. This complex system not only plays a role in cell-to-cell communication but also synchronizes the provision of energy for neural networks. Astrocytes contain glycogen, a form of energy store. Excitation of astrocytes by volume transmitters, such as noradrenaline (NA), released by locus coeruleus neurons, activates adrenergic receptors and stimulates glycogenolysis, providing lactate. This lecture will discuss how astrocytes operate to synchronize excitation and energy provision. Moreover, Ca2+-dependent fusion of the vesicle membrane with the plasma membrane in astrocytes will be presented.
Supported by Slovenian Research Agency, CipKeBip, EduGlia.

Plenary Speakers

Neuroimmune transformation in Parkinson's disease

Howard E. Gendelman and R. Lee Mosley

Department of Pharmacology and Experimental Neuroscience, University of Nebraska Medical Center, USA

Neuroimmune Pharmacology of GM-CSF Until now therapeutic interventions that serve to modulate inflammatory responses, protect neurons and clear misfolded and aggregated proteins have failed to demonstrate clinical benefit for Parkinson's disease (PD) or for other neurodegenerative disorders. This includes a spectrum of immunization regimens designed to clear misfolded proteins. Such misfolded proteins occur either as a random event over time or a change in the brain's microenvironment. Clearance of these oxidative-modified misfolded proteins, such as α-synuclein, β-amyloid, and tau requires a change in activation profiles of mononuclear phagocytes (MP; monocyte, macrophages and microglia) potentiated, in measure, by CD4+ effector T cells (Teff). However, dysregulation of Teff and MP results in aberrant neuroinflammation and induction of aggressive proinflammatory immunity that perpetuate a cycle of inflammation - protein misfolding - neurodegeneration. This can be perpetuated by immunization with modified or misfolded proteins. We posit that immune activities that occur in the brain's microenvironment during disease are transformable as neurotoxic proinflammatory activities were reconverted into neuroprotective responses through the induction of CD4+ regulatory T cells (Treg), however the two-way plasticity of Teff and Treg depending on the pro- or anti-inflammatory cytokine microenvironment may critically affect inflammation status, clearance of proteins, and neurodegenerative processes. For instance, we showed the administration of granulocyte macrophage-colony stimiulating factor (GM-CSF) attenuated neuroinflammation and spared dopaminergic neurons in the 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine (MPTP) model of PD. Moreover, GM-CSF upregulated Treg function, which upon adoptive transfer into MPTP mice were also anti-inflammatory and

neuroprotective. We showed a synthetic vasoactive intestinal peptide receptor-2 agonist shown to mediate anti-inflammatory and neuroprotective functions, and also induced increase in expression of GM-CSF by CD4+ T cells. In a recently completed Phase I clinical trial of PD patients, we demonstrated that sargramostim (GM-CSF) increased Treg function and diminished Teff frequency with parallel improvements in motor functions as assessed by the Unified PD Rating Scores (ClinicalTrials.gov number, NCT01882010). The results of this clinical trial including biomarker discoveries made will be discussed

Alexej Verkhratsky title: "Reassessing neurocentrism: Principles of astrogliopathology"

REASSESSING NEUROCENTRISM: PRINCIPLES OF ASTROGLIOPATHOLOGY. Verkhratsky, A, MD, Ph.D. [1]; [1]The University of Manchester, Faculty of Life sciences, Manchester, M13 9PT.

The common and prevailing set of neurological thoughts considers neurones as the primary substrate of pathological progression. This "neurone-centric" concept, however, undergoes a dramatic change. It has become universally acknowledged that integration and information processing in the brain occurs though close interactions of synaptically connected neuronal networks and complex fabric of neuroglial cells. There is compelling evidence demonstrating that astrocytes create the compartmentalisation in the CNS, and integrate neurones, synapses, and brain capillaries into individual and relatively independent units. Astroglial syncytia allow intercellular communication, accomplished through translocation of ions, metabolic factors and second messengers. Many levels of integration, both morphological and functional, presented by neuronal-glial circuitry ensure the spatial and temporal multiplication of brain cognitive power. Neuroglial cells contribute to all forms of neurological diseases and glial reactions, determine, to a very large extent, the progression and outcome of neuropathology. Astrocytes are specifically involved in various neurodegenerative diseases including Alzheimer's disease, Amyotrophic lateral sclerosis, Parkinson's disease and various forms of dementia. Astrocytes undergo both atrophy and reactivity, which are specific for different stages of the disease evolution. Astroglial reactivity represents the generic defensive mechanism, and inhibition of astrogliotic response often exacerbates neuropathology.

GENERAL ABSTRACTS

PERIPHERAL EFFECTS OF THE INTERACTION BETWEEN ALCOHOL AND THE HDAC INHIBITOR MOCETINOSTAT. Agudelo, M, Ph.D. 1, Figueroa, G, BS 1, Parira, T, BS 1, Laverde, A 1, Madhavan , N, Ph.D. 1; 1Herbert Wertheim College of Medicine, Florida International University, Miami, FL 33190.

Epigenetic mechanisms have been shown to play a role in alcohol use disorders (AUDs) and may prove to be valuable therapeutic targets. In addition, histone deacetylase inhibitors (HDACi) have been shown to have anti-inflammatory and neuroprotective effects. However, HDACi have been also known to mediate the induction of apoptosis and autophagy . In an effort to elucidate the role of HDACi and their modulation of alcohol-induced effects in the periphery, we proceeded to test whether the interactions of EtOH with HDACi, mocetinostat (MGCD0103), are affecting antioxidant responses and inducing apoptosis in monocyte derived dendritic cells (MDDCs). Oxidative stress and apoptosis related genes were measured in vitro using PCR array and and in silico using GNCPro Gene Network Central

research tool. Our results demonstrated that mocetinostat induced a dramatic increase on antioxidant responses, ROS metabolism genes, and apoptosis related genes through the regulation of class I HDACs as confirmed by western blot. In silico analysis revealed new target genes and pathways on the mode of action of alcohol and HDAC inhibitors. Findings elucidating the interaction of HDAC inhibitors with alcohol and their effects on oxidative stress and apoptosis may be useful for the development of novel treatments for alcohol-induced oxidative damage and may delineate new immune-modulatory mechanisms.

Supported by NIAAA R00AA021264.

DNA DAMAGE IN HIV-1 PATIENTS TAKING ART. Albino, EM, Ph.D. 1, Figueroa, L 2, Van Daalen, Y 2, Godoy, L, BS 1, Hill, M, Ph.D. 1, Rivera-Amill, V, Ph.D. 3; 1Pharmacology and Toxicology Department, Ponce Health Sciences University, Ponce, PR 007322Biology Department, University of Puerto Rico in Ponce, Ponce, PR 007323Microbiology Department, Ponce Health Sciences University, Ponce, PR 00732.

Despite the reduction in mortality in HIV-1-infected patients due to antiretroviral therapy (ART), adverse effects have been associated to long-term exposure to treatment. Recent studies have demonstrated that patients infected with HIV-1 are under chronic oxidative stress. However, more studies are required to determine whether the oxidative stress is due to the HIV-1 infection or to the adverse effects antiretroviral therapy. Oxidative stress induces structural and functional changes to lipids, proteins, and DNA. Single-strand breaks (SSBs) are the most common DNA damage in cells. The aim of this study is to evaluate the DNA damage in peripheral blood mononuclear cells (PBMCs) from HIV-1 infected subjects treated with ART. DNA damage was compared in untreated HIV-1 positive subjects, HIV-1 positive under ART, HIV-1 negative under pre-exposure prophylaxis (PrEP) and healthy donors. SSBs were evaluated using an alkaline gel electrophoresis and an alkaline comet assay. PBMCs from healthy donors were used as negative controls while ethylmethane sulfonate (EMS) treated PBMCs were used as a positive control. Interestingly, a higher DNA fragmentation in HIV-1 positive samples taking ART was observed when compared to the HIV-1-negative control and to EMS treated positive control. The extent of the SSBs varied among samples depending on type of therapy and the length of time under treatment. Taken together, our results suggest that ART may affect the oxidative stress imbalance in HIV-1 positive patients.

UTILITY OF LIF TO PROTECT AGAINST HIV-1 ASSOCIATED NEUROCOGNITIVE DISORDERS. Alves, J.M., Ph.D. 1, Hunter, R.M., MD, Ph.D. 1, Cruz, M.L., MS 2, Ramos, K., BS 1, Velázquez, B., BS 2, Noel, R., Ph.D. 2; 1Microbiology and Immunology Department, Universidad Central del Caribe, Bayamón, PR 009602Basic Sciences Department, Ponce Health Sciences University, Ponce, PR 00716.

The therapy of HIV/AIDS has expanded over the years to include 26 drugs. However, rapid development of drug resistant-viral strains makes antiretroviral therapy for HIV infection transiently effective in some scenarios, in others the concept of stable quiescent CD4 T cell, astrocytes and microglia functioning as reservoirs of the latent infection represent the major hurdle towards the cure of the infection. Prolonged human immunodeficiency virus-1 (HIV-1) infection leads to neurological debilitation, including motor dysfunction and frank dementia. Although pharmacological control of HIV

infection is now possible, HIV-associated neurocognitive disorders (HAND) remain intractable. In addition, current antiretroviral drugs have low bioavailability in the brain and no antiviral drug is currently available that can silence or eliminate the DNA of HIV provirus from reservoirs within the central nervous system. Therefore, an alternative approach should be found to prevent or alleviate the CNS damage following HIV-1 infection. In this regard, we propose to use the supernatant of neural progenitor cells (NPC) more specifically secreted factors by these cells known as leukemia inhibitor factor (LIF). The NPC are particularly interesting because correspond a complex microenvironment capable to release factors to induce differentiation and give origin to neurons, astrocytes and oligodendrocytes. Therefore, this project will examine the hypothesis that the LIF treatment will protect against HIV/Nef-mediated neuronal damage due to anti-inflammatory and importance neuroprotective factors.

Supported by Puerto Rico Clinical and Translational Research Consortium (PRCTRC-NIH) Grant Number: U54 MD007587.

DECLINE OF SIRTUIN-1 EXPRESSION/ACTIVITY PLAYS CRITICAL ROLE IN BRAIN ENDOTHELIAL DYSFUNCTION IN CAA-VASCULOPATHY. Andjelkovic, AV, MD, Ph.D. 1, Stamatovic, SM, MD, Ph.D. 1, Keep, RF, Ph.D. 2; 1Department of Pathology, University of Michigan, Ann Arbor, MI 481092Department of Neurosurgery, University of Michigan, Ann Arbor, MI 48109.

Cerebral amyloid angiopathy (CAA), a degenerative neurovascular disease characterized by the progressive deposition of amyloid-beta within walls of cerebral microvessels and associated neuroinflammation, is critical component leading to cognitive impairment in age-associated dementia. The brain endothelial dysfunction is considered to have critical role in pathogenesis of CAA. In our parallel PCRarray and proteomic analysis of brain microvessels from patients with different grade of CAA and mice with murine model of capillary CAA (Tg-SwDI and Tg-SwDI/NOS2−/−) we have found significant decreased in Sirt1 expression in brain endothelial cells over the course of disease. SIrt1 deprivation was directly associated with alteration in brain endothelial barrier integrity (hyperpermeability) due to reorganization of actin cytoskeleton and decreased expression of actin binding protein VASP and alteration of endothelial phenotype in proinflammatory (increased expression of cytokines IL16 and adhesion molecules ICAM-1 and JAM-A). As potential underlying mechanisms we identified that Sirt1 depletion activated NFkB, increased acetylation of NFkBp65 units, which in turn represses VASP expression and alter barrier integrity and could also regulated activity of DNA methyltransferases, causing the decreased methylation of IL-16 and JAM-A CpG sites and increased IL-16 and JAM-A expression. Administration of Sirt1 agonist SRT1720 in Tg-SwDI mice exerted anti-inflammatory and brain endothelial barrier stabilizing effect. Our result indicated Sirt1 as potential target in CAA treatment.

Supported by NIH /NS062853 (A.V.A).

DECLINE OF SIRTUIN-1 EXPRESSION/ACTIVITY PLAYS CRITICAL ROLE IN BRAIN ENDOTHELIAL DYSFUNCTION IN CAA-VASCULOPATHY. Andjelkovic, AV, MD, Ph.D. 1, Stamatovic, SM, MD, Ph.D. 1,

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Supported by NIH /NS062853 (A.V.A).

EXTRACELLULAR VESICLES OF THE BLOOD-BRAIN BARRIER: ROLE IN THE HIV-1-INDUCED AMYLOID BETA PATHOLOGY. Andras, IE 1, Leda, A 1, Garcia Contreras, M 2, Bertrand, L 1, Skowronska, M 1, Toborek, M 1; 1Department of Biochemistry and Molecular Biology, University of Miami School of Medicine, Miami, FL 331362Diabetes Research Institute, University of Miami School of Medicine, Miami, FL 33136.

HIV-infected brains are characterized by increased amyloid beta (Aβ) deposition. It is believed that the blood-brain barrier (BBB) is critical for Aβ homeostasis and contributes to Aβ accumulation in the brain. Our previous data indicated that exposure to HIV-1 can increase Aβ levels in brain endothelial cells and elevate its transendothelial transfer via the involvement of lipid rafts and the Ras-MAPK signaling pathway. Recently, extracellular vesicles (ECV) gained a lot of attention as potentially playing a significant role in neurodegenerative diseases, particularly in Aβ pathology. In addition, HIV-1 can hijack the exosomal pathway leading to increased viral spread. Therefore, we investigated the involvement of BBB-derived ECV in the HIV-1-induced Aβ pathology of the brain. Our data have shown that HIV-1 increased ECV release from brain endothelial cells and elevated ECV Aβ, which could be taken up by astrocytes, indicating that ECV can transfer Aβ between different cell types of the BBB. Infusion of brain endothelial ECV carrying fluorescent Aβ into the internal carotid artery of mice resulted in Aβ fluorescence associated with brain microvessels. Based on these observations, we conclude that HIV-1 facilitates shedding of brain endothelial ECV carrying Aβ, therefore increasing Aβ exposure of the neighboring cells of the brain microvessels. This mechanism may contribute to neurovascular unit dysfunction and the development of HIV associated neurocognitive disorders (HAND).

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COMPARISON OF THE EFFECTS OF PSYCHOSTIMULANT DRUGS OF ABUSE ON BRAIN ENDOTHELIAL BARRIER INTEGRITY AND EXTRACELLULAR MICROVESICLE PRODUCTION. Andrews, AM, Ph.D. 1, Merkel, SF, MS 1, Lutton, EM, BS 1, Rawls, SM, Ph.D. 2, Ramirez, SH, Ph.D. 1; 1Department of Pathology & Laboratory Medicine, Lewis Katz School of Medicine at Temple University, philadelphia, PA 191402, The Center for Substance Abuse Research, Lewis Katz School of Medicine at Temple University, Philadelphia, PA 191403The Shriners Hospitals Pediatric Research Center, Shriners Hospital for Children, Philadelphia, PA 19140.

The blood brain barrier (BBB) regulates homeostasis and supports metabolic activity in the central nervous system. Although this complex barrier is comprised of multiple cell types, endothelial cells are primarily responsible for physically forming the barrier through the formation of tight junctions. Research has shown that psychostimulants alter BBB function and increase neuroinflammation. However, how the various psychostimulant drugs specifically affect the cerebral endothelium has not been fully investigated. We have compared the effects of cocaine, methamphetamine and 'bath salt' designer cathinones (MDPV) on cerebral endothelial integrity, adhesion molecule expression and production of extracellular microvesicles (eMV). Both cocaine (100 μM) and methamphetamine (50 μM) disrupted BBB integrity and upregulated adhesion molecule expression. In addition, both induced the production of eMVs containing tight junction proteins as a result of a BBB breakdown. In the case of cocaine, increased eMV production occurred at concentrations lower than what was needed to disrupt the barrier and upregulate adhesion molecule expression. On the other hand, synthetic cathinones, which have been reported to be 10 times more potent than other psychotimulants, had no effect on barrier integrity, adhesion molecule expression or production of eMVs. Overall we have found that psychostimulants have different effects, which likely plays an important role in the progression of drug-induced BBB dysfunction and neuroinflammation.

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HIV-1 SUBTYPE B TAT AMINO ACID SUBSTITUTIONS ASSOCIATE WITH NEUROCOGNITIVE IMPAIRMENT IN THE DREXEL MEDICINE CARES COHORT. Antell, G, BS 1, Pirrone, V, Ph.D. 2, Dampier, W, Ph.D. 2, Aiamkitsumrit, B, Ph.D. 2, Williams, J, BS 2, Passic, S, MS 2, Kercher, K, MS 2, Zhong, W., BS 2, Jacobson, J, MD 3, Wigdahl, B, Ph.D. 1, Hershberg, U, Ph.D. 1, Nonnemacher, M, Ph.D. 2; 1School of Biomedical Engineering, Science and Health Systems, Drexel University, Philadelphia, PA 191042Microbiology and Immunology, Drexel University College of Medicine, Philadelphia, PA 191023Medicine, Division of Infectious Disease and HIV Medicine, Drexel University School of Medicine, Philadelphia, PA 19102.

The current studies seek to identify and characterize amino acid variations within the HIV-1 protein Tat in association with neurocognitive impairment and their anatomical source. HIV-1 Tat sequences were obtained from both the Drexel Medicine CNS AIDS Research and Eradication Study (CARES) Cohort as well as from autopsy brain tissue obtained from the National NeuroAIDS Tissue Consortium (NNTC).

Sequences acquired from the CARES Cohort were amplified from peripheral blood mononuclear cells, 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 HIV-1 subtype B consensus reference genome in order to compare amino acid substitutions. Comparisons were made on the basis of anatomical compartment and degree of neurocognitive impairment as assessed using both a Modified Hopkins Dementia Score (MHDS) and a Global Deficit Score (GDS). Multiple positional hotspots of high variation in Tat have been identified within the cysteine-rich domain, glutamate-rich domain, and second exon. In contrast, the TAR-binding domain was highly conserved. Statistical analyses were applied to both amino acid positions and amino acid variants associated with neurocognitive impairment status. Overall, these analyses have resulted in the identification of compartmentalization between brain and PBMC-derived sequences, as well as Tat sequence variants specific for patients in the absence or presence of neurocognitive impairment, which may prove useful in further characterization of HIV-1 neuropathogenesis.

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COCAINE-INDUCED MICROGLIA ACTIVATION IN NUCLEUS ACCUMBENS AND CAUDATE-PUTAMEN IS REVERSED BY NALOXONE. Avalos, MP, MS 1, Mongi-Bragato, B, Ph.D. 1, Bartos, M, Ph.D. 3, Iribarren , P, Ph.D. 2, Cancela, LM, Ph.D. 1; 1Institute of Experimental Pharmacology CONICET, School of Chemistry Sciences, Cordoba, 50162Institute of Biochemist and Immunology CONICET, School of Chemistry Sciences, Cordoba, 50163School of Biochemist, University of South, Bahia Blanca, 5016.

Previous findings from our lab show a long-lasting psychostimulant-induced sensitization phenomenon at the immune level in a manner parallel to that occurring in the limbic and immune enkephalinergic systems (Assís et al., 2006, 2009, 2011). We also demonstrated that the enkephalinergic system is essential for behavioral and molecular sensitization to cocaine within the nucleus accumbens (NAc) and caudate putamen (CPu) (Mongi-Bragato et al., 2014). However, there is no description so far of how microglia is involved in psychomotor sensitization and how the enkephalinergic system participates in this. We treated male C57B/6J mice daily with naloxone (1 mg/kg i.p.) or vehicle previous to cocaine (15 mg/kg i.p.) and vehicle for 9 days, followed by a cocaine challenge (7.5 mg/kg i.p.) on day 21 of the treatment. The immunohistochemistry was performed in the areas of interest using CD11b and met-ENK antibodies. Cytokines were measured by qRT-PCR. In the control treatment, microglia cells had small soma and ramified processes. Repeated administration of cocaine induced morphological changes of microglial cells indicative of cell activation and also increased production of pro-inflammatory cytokines such as TNF-α, which was reversed by naloxone pretreatment. These preliminary results could be key to a better understanding of the role of the enkephalinergic system in the immunological signaling system in drug addiction and may provide a new therapeutic target.

Supported by FONCyT, CONICET, SECyT.

ARGONAUTE AND DICER-MEDIATED DYSREGULATED EXPRESSION OF MIRNAS IN THE PBMCS FROM PTSD PATIENTS. Bam, Marpe, Ph.D. 1, Nagarkatti, Prakash, Ph.D. 1, Nagarkatti, Mitzi, Ph.D. 1; 1Pathology Microbiology and Immunology, University of South Carolina, Columbia, SC 29209.

PTSD is a psychiatric disorder with patients experiencing chronic systemic inflammation and dysregulated expression of IFNG, IL12 etc., in PTSD is already reported. However, regulation of these genes is poorly understood. Role of miRNAs is one of the key mechanisms of gene regulation. A defect in miRNA biosynthesis can result in dysregulation of the miRNAs which can impact the regulation of protein coding gene expression. By RNA-seq, we observed that AGO2 and Dicer 1 expression is lower in the PBMCs of PTSD. Both AGO2 and DICER1 are responsible for the generation of mature miRNAs. Furthermore, by miRNA microarray we observed a massive downregulation of miRNAs in the PBMCs from PTSD patients. Thus, we hypothesized that lower expression of AGO2 and DICER1 occurs during PTSD and results in the downregulated expression of miRNAs. Target analysis revealed that several of the pro-inflammatory and related genes are targets of the downregulated miRNAs. Thus, we further hypothesized that dysregulated expression of the immune system genes during PTSD is contributed by defective miRNA biogenesis. Knockdown experiments showed that the expression of several dysregulated miRNAs is altered as a result of the knockdown of AGO2 and DICER1 independently. Our findings show a clear future direction for studies to understand more about the inflammation seen in PSTD. The present data further provide strong evidence that PTSD manifests an inflammatory condition which is epigenetically regulated and this can be employed as biomarkers for PTSD diagnosis and combating the chronic inflammation.

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DISRUPTION OF TIGHT JUNCTION INTEGRITY IN BRAIN MICROVASCULAR ENDOTHELIUM BY ANTI-RETROVIRAL THERAPY. Bertrand, L, Ph.D. 1, Dygert, L 1, Toborek, M, MD, Ph.D. 1; 1Biochemistry and Molecular Biology, University of Miami, Miami, FL 33136.

The project aims at identifying the impact of cerebrovascular toxicity mediated by antiretroviral drugs (ARVd) used in the treatment of HIV on the integrity of the blood-brain barrier (BBB) and the development of stroke. We recently (Bertrand and Toborek, Mol Pharmacol, 2015) demonstrated a new mechanism of endothelial toxicity for the ARVd Efavirenz, a non-nucleoside reverse transcriptase inhibitor (NNRTI), based on dysregulation of ER stress and apoptosis responses. Since the disruption of these cellular responses is likely to affect other cellular function; our hypothesis is that Efavirenz toxicity affects brain endothelium integrity and lead to an increase in blood-brain barrier permeability. Indeed, exposure to this ARVd resulted in an increase in monolayer permeability. To explain this outcome, we evaluated the expression of three major tight junction proteins: Claudin-5, ZO-1 and Occludin. A specific downregulation of Claudin-5 was observed following exposure to Efavirenz. Furthermore, when the localization of these proteins was analyzed, we observed that Claudin-5 is absent from cell-cell contact area following Efavirenz exposure, but ZO-1 and Occludin are not affected. These changes were also observed in isolated microvessels from mice treated with this ARVd. Because tight junctions are primordial to the function of the BBB, these results indicate that Efavirenz toxicity may be a major contributor to the increase in BBB permeability and affect the development of neurodegenerative diseases in HIV patients.

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REGULATION OF ASTROCYTE-TRACE AMINE ASSOCIATED RECEPTOR 1 SUBCELLULAR DISTRIBUTION AND INTERACTING PARTNERS IN THE CONTEXT OF METHAMPHETAMINE AND HIV-ASSOCIATED NEUROCOGNITIVE DISORDERS. Borgmann, K, BS 1, Ghorpade, A, Ph.D. 1; 1Cell Biology and Immunology, University of North Texas Health Science Center, Fort Worth, TX 76107.

As a psychostimulant, methamphetamine (METH) use leads to long-lasting, euphoric effects. Between 10- 15% of human immunodeficiency virus-1 (HIV-1) patients report METH abuse, which exacerbates HIV-1 infection, accelerating the onset of HIV-associated neurocognitive disorders (HAND) and immune dysfunction. Neuroinflammation, glial activation, oxidative stress and excitotoxicity contribute to METH and HIV neuropathogenesis. However, the mechanisms through which METH and HIV affect astrocyte function are unclear. Recently, we reported trace amine associated receptor 1 (TAAR1) as a novel astrocyte receptor for METH. Previous studies suggest TAAR1 activity may be regulated by G-protein promiscuity and desensitization by β-arrestin. We hypothesize that HIV-relevant stimuli upregulate astrocyte-TAAR1 expression and that METH exposure induces alterations in TAAR1 activation and intracellular localization, thus contributing to astrocyte dysfunction. To examine subcellular distribution TAAR1 expression was assessed by confocal microscopy in human astrocytes in the context of HIV and METH exposure. Changes in EAAT2, which could impair astrocyte ability to clear glutamate, were examined in parallel. To assess TAAR1 regulation by interacting partners, co-immunoprecipitation studies were performed, specifically to investigate β-arrestin activation and TAAR1-mediated calcium signaling via Gαq. These studies delineate how dysregulation of TAAR1 may contribute to astrocyte-mediated neurodegeneration during HAND and METH abuse, while also revealing a novel therapeutic target in astroglia.

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CORIOLUS VERSICOLOR'S POLYSACCHARIDE PEPTIDE ACTIVATES THE IMMUNE RESPONSE AND INHIBITS HIV-1 REPLICATION. Boukli, Nawal, Ph.D. 1, Rodríguez, M, MS 1, López, S, MS 1, Rivera, M, MS 1, Rodríguez, M, MS 1, Cubano, L, Ph.D. 1, Ríos, E, Ph.D. 1; 1Biomedical Proteomics Facility, Microbiology and Immunology Department, Universidad Central del Caribe School of Medicine, Bayamon, PR 00960.

The Medicinal fungus Coriolus versicolor has proven its benefits in many clinical trials in Asia. An active principle of Coriolus, Polysaccharide Peptide (PSP) has demonstrated to be an immune system builder and has been shown to exhibit antiviral and immunomodulatory properties. Because highly active antiretroviral therapy are still relatively toxic and expensive to be afforded by most HIV patients, there is a major need to identify compounds that can prevent the adverse effect occurring post-HIV-1 infection. In vitro cytotoxicity and dose-response assays were conducted to evaluate PSP anti-HIV-1 activity on Peripheral Blood Mononuclear Cells (PBMC). The results showed that PSP was non-toxic in human PBMC at 200ug/ml. p24 ELISA assay was used to assess HIV replication which was inhibited by 48.9 % and 72.6 % after respectively one and two administrations of 200ug/ml PSP during 3 and 6 days treatment in PBMC. Moreover, PSP has demonstrated its anti-HIV and immune boosting capabilities by upregulating RANTES and MIP-b. PSP treated PBMC in vitro were also able to induce NFκβ, Dectin-1 and

TLR 2 and 4 production, suggesting activation of ROS/oxidative stress leading to the pro-inflammatory mediators expression described above as well as PSP induced expression of functional innate immune receptors. Further studies are in progress to understand the molecular mechanisms behind PSP anti-HIV activity and to prevent further spread of HIV-1 infection and disease progression.

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USING CRISPR/CAS9 TO CREATE HIV-NANOLUC CHME-5, A NOVEL MICROGLIA CELL LINE. Campbell, LA, Ph.D. 1, Ritchie, C, Ph.D. 1, Heathward, E, MS 1, Harvey, BK, Ph.D. 1; 1Optogenetic and Transgenic Technology Core, National Institute on Drug Abuse, Baltimore, MD 21224.

Microglia play a key role in the pathogenesis of Human Immunodeficiency Virus (HIV)-associated neurocognitive disorders (HAND) due to their productive infection by HIV. This results in the release of neurotoxic viral proteins and pro-inflammatory compounds which hinder the functionality of surrounding neurons. Because models of HIV infection within the brain are limited, we aimed to create a novel microglia cell line capable of recreating several hallmarks of HIV infection. We utilized CRISPR/Cas9 gene editing technology to integrate a modified HIV provirus into CHME-5 immortalized microglia to create HIV-nanoLuc CHME-5. In the modified provirus, the Gag-Pol region is replaced with a luciferase; nanoLuc, which allows for rapid assay of HIV long terminal repeat (LTR) activity using a luminescent substrate. HIV-nanoLuc CHME-5 microglia were stimulated with lipopolysaccharide (LPS), resulting in elevated nanoLuc expression, along with mRNA and protein production of HIV viral products Nef and gp120. Stimulation also released the chemokines CXCL-2 and CXCL-3, suggesting that CRISPR/Cas9 genome editing did not alter the microglia phenotype. Furthermore, we co-cultured HIV-nanoLuc CHME-5 with primary cortical neurons expressing the calcium indicator GCaMP to examine their effects on neuronal activity. We observed a change from synchronous neuronal firing in control conditions to sporadic single cell firing with addition of HIV-nanoLuc CHME-5. Overall these data suggest that HIV-nanoLuc CHME-5 may be a novel tool to enhance the study of HIV mediated neuropathology.

IMPACT OF ACUTE HYPOXIA AND GRADED EXERCISE TO VOLITIONAL EXHAUSTION ON CIRCULATING BDNF LEVELS AND PSYCHOMOTOR PERFORMANCE IN YOUNG HEALTHY SEDENTARY MALES. Chalimoniuk , M, Ph.D. 1, Czuba , M, Ph.D. 2, Langfort , J, Ph.D. 3; 1The Department of Tourism and Health in Biała Podlaska, Józef Piłsudski University of Physical Education, Warsaw, 40-065 2Department of Sports Theory, The Jerzy Kukuczka Academy of Physical Education, Katowice, 40-065 3Department of Physiology, The Jerzy Kukuczka Academy of Physical Education, Katowice, 40-065 .

Exercise performance involving large muscle groups is lower in hypoxia than in normoxia and this phenomenon may be attributed to muscle fatigue. An alternative hypothesis that may partially explain reduced exercise performance in hypoxic condition is so-called central fatigue. To examine such a possibility, we investigated the effect of exercise to volitional exhaustion (EVE) performed in hypoxia and normoxia on circulating BDNF levels and on the multiple choice reaction time (MCRT, an index of psychomotor performance) during the incremental cycloergometer test (ICT). An impact of hypoxia on participants' organism was indicated by measurement of EPO levels. Ten young male individuals (20-30

yrs) were randomly divided to complete the ICT both in normoxia and two hypoxic conditions, one was an equivalent of 2000 m (16.6% O2; Hyp-2) altitude and the second an equivalent of 3000 m (14.7 % O2; Hyp-3) altitude. An increase in serum EPO levels was detected during EVE in both hypoxic conditions. However, distinct responses on serum BDNF levels were observed during EVE in normoxia and Hyp-2 as 6 participants shown a decrease and 4 an increase of this variable. Under Hyp-3, the responses were uniformed and an increase in BDNF level was observed in all participants. The MCRT did not change in any of the studied group. These findings indicate that exposition to acute hypoxia of young healthy individuals does not affect their psychomotor performance but shows diversified responses in BDNF production, which may be related to central mechanisms and/or the blood brain barrier status.

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THE EFFECT OF AUTOLOGOUS TRANSPLANTATION OF ADIPOSE STEM CELLS ON MODULATION OF IMMUNE AND INFLAMMATORY RESPONSES IN SM PATIENTS. Chalimoniuk, M, Ph.D. 1, Lubina-Dabrowska, N, Ph.D. 2, Machaj, EK, Ph.D. 3, Langfort, J, Ph.D. 4, Pojda, Z, MD, Ph.D. 5, Stepien, A, Ph.D. 2; 1Department of Cellular Signaling, Mossakowski Medical Research Centre, Warsaw, 02-1062Department of Neurology, Military Institute of Medicine, Warsaw, 04-1413Department of Breast Cancer and Reconstructive Surgery, M. Sklodowska-Curie Memorial Cancer Center and Institute of Oncology, Warsaw, 02-0344Department of Pharmacology, Mossakowski Medical Research Centre, Warsaw, 02-1065Laboratory of Cellular Engineering, M. Sklodowska-Curie Memorial Cancer Center and Institute of Oncology, Warsaw, 02-034.

The aim of our study was to investigate the immunomodulation of immune system after autologous transplantation of adipose stem cells (ASC) in patients with MS. ASC have the same therapeutic properties as myeloid mesenchymal stem cells (MSC) but their additional therapeutic advantage relies on the high purity of the probe and very safe technique of obtaining them from a donor. Sixteen patients with Relapsing-Remitting MS were enrolled in the study. Adipose tissue was collected according to the Coleman's technique. ASC cells were injected intrathecally (12 x 106 cells/dose) at the enrollment. The injections were repeated at the 3rd and the 12th month of therapy with cryopreserved ASC. IL-6, IL17 and IL10 and the efficacy parameters (EDSS, MS Functional Scales cores, relapse incidents, MRI lesion burden, and the whole brain and gray matter atrophy rates) were monitored throughout 12 month period. The serum levels of assessed inflammatory cytokines (IL-6, IFN-γ, TNF-α) and spinal cord fluid of IL17 decreased in serum after 12 months of ASC therapy. The therapy also decreased prooxidative factors, such as 4-hydroxynonenal and nitrite. Neurological examination performed in SM patients after the ASC injection did not reveal any adverse effects while their neurological status examined by EDSS improved. There were no relapses during an entire period of therapy. While the initial results presented herein are promising, this therapeutic approach requires a larger number of examined patients and a longer observation time.

THE INFLUENCE OF CHRONIC VENLAFAXINE ADMINISTRATION ON THE CHEMOKINE CXCL12 CONCENTRATION IN THE BRAIN OF ADULT OFFSPRING RATS – STUDY IN THE ANIMAL MODEL OF DEPRESSION. Chamera, K., MS 1, Trojan, E., MS 1, Ślusarczyk, J., MS 1, Głombik, K., Ph.D. 1, Basta-Kaim,

A., Ph.D. 1; 1Department of Experimental Neuroendocrinology, Institute of Pharmacology Polish Academy of Science, Kraków, 31-343.

The changes in expression of chemokines in CNS can underlie pathological processes. Particularly interesting are the data concerned the ability of CXCL12 to modulate serotonergic transmission, so chemokines may be involved also in antidepressants action. The aim of this study was to examine the impact of prenatal stress procedure on the behavioral and biochemical concentration of CXCL12 in frontal cortex and hippocampus of adult offspring rats. Moreover the effect of chronic treatment of venlafaxine on above-mentioned parameters in the animal model of depression was evaluated. Pregnant rats were subjected to restraint stress until the delivery. 3-month-old rats were tested for behavioral changes in forced swim test. Therefore male offspring was administered i.p. for 14 days with venlafaxine (10 mg/kg) or appropriate vehicle. The animals' behavior was tested again and 24 hours later rats were sacrificed to determine the level of CXCL12 in both brain areas by ELISA assays. The study confirmed that adult offspring rats after prenatal stress procedure exhibit behavioral disturbances. Biochemical study showed significantly higher level of CXCL12 both in frontal cortex and hippocampus of prenatally stressed offspring. The chronic venlafaxine treatment normalized the behavioral changes. Our study showed that prenatal stress procedure leads to persistent behavioral and biochemical disturbances in adult offspring rats. It may be postulated that the therapeutic efficacy of venlafaxine is related to normalization of some chemokines levels in the animal model of depression.

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METHAMPHETAMINE ALTERS FUNCTIONAL ACTIVITY OF POTASSIUM CHANNELS THROUGH THE TAAR1-MEDIATED SIGNALING PATHWAY IN PRIMARY HUMAN FETAL ASTROCYTES. Chen, L, Ph.D. 1, Dave, S, Ph.D. 1, Yu, C, Ph.D. 2, Seaton, M, BS 2, Khodr, C, Ph.D. 1, Al-Harthi, L, Ph.D. 2, Hu, X-T, MD, Ph.D. 1; 1Department of Pharmacology, Rush University Medical Center, Chicago, IL 606122Department of Immunology and Microbiology, Rush University Medical Center, Chicago, IL 60612.

Methamphetamine (Meth) is a potent and commonly-abused psychostimulant. Chronic exposure to Meth decreases neuronal activity in certain brain regions that are key regulator of cognition and addiction, and that may contribute to mechanism underlying Meth addiction. It is not fully understood whether such decrease results from altered synaptic/intrinsic excitability of neurons, and/or dysregulation of the extracellular environment (e.g., glutamate and K+ levels) mediated by astrocytes. To determine the effects of Meth on K+ regulation by astrocytes, we assessed the activity of various K+ channels in cultured primary human fetal astrocytes (HFAs) using whole-cell patch-clamp recording. We found that activated HFAs displayed large, outflowing voltage-gated K+ currents (IKv, characteristic of immature or reactive astrocytes) and moderate inwardly-flowing K+ currents (IKir). Meth depolarized the resting membrane potential (RMP), reduced IKv, but had no significant effect on IKir in HFAs. Blockade of the two-pore domain K+ (K2P) channels (by quinidine) and IKv (by TEA) mimicked the Meth effects on RMP and IKv efflux, respectively. Moreover, inhibition of the trace amine-associated receptor 1 (TAAR1, by EPPTB) and PKA-like activity (by H-89) abolished the Meth effects on IKv. These findings suggest that Meth disturbs HFA function by reducing functional activity of K2P and Kv channels through facilitation of the TARR1/PKA signaling pathway. These changes could consequently reduce local extracellular K+ level, and ultimately decrease excitability of surrounding neurons in the brain.

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STREPTOZOTOCIN AND DIMETHYL FUMARATE-INDUCED CHANGES IN PLASMA CORTICOSTERONE CONCENTRATION IN OLD RATS WITH DIFFERENT BEHAVIORAL CHARACTERISTICS. Chomik, Aleksandra 1, Podlacha, Magdalena 1, Majkutewicz , Irena 1, Wrona, Danuta 1; 1 Department of Animal and Human Physiology, University of Gdansk, Gdansk, 80-309.

The study examined an effect of intracerebroventricular (i.c.v.) injection of streptozotocin (STZ), model of sporadic Alzheimer's Disease (sAD) and dimethyl fumarate (DMF), anti-inflammatory drug, on plasma corticosterone (CORT) response in old (24 mounth-old) Wistar rats, differing in locomotor response to novelty/stress susceptibility (high (HR) or low (LR) responders). HRs and LRs were divided into groups: STZ DMF (i.c.v. injection of STZ, 0.4% DMF fodder), VEH DMF (i.c.v. injection of vehicle, 0.4% DMF fodder), STZ CTR (i.c.v. injection of STZ, standard fodder), VEH CTR (i.c.v. injection of vehicle, standard fodder). 21 days after STZ injection and feeding with DMF, blood samples were taken for determination of plasma CORT concentration (RIA). STZ significantly ($p \leq 0.05$) decreased plasma CORT concentration in the non-divided behaviorally STZCTR group (147.45±69.05 ng/ml) as compared to the VEHCTR (219±64.69 ng/ml) whereas it increased CORT response in the STZDMF group (153.39±63.84 ng/ml, $p \leq 0.01$; HRs: 199.67 ng/ml, LRs: 140.64±57.73 ng/ml, $p \leq 0.05$) as compared to the control VEHDMF rats (63.37±33.86 ng/ml; HRs:103.12±34.43 ng/ml; LRs:40.64±12.87 ng/ml). DMF decreased plasma CORT level within the non-divided ($p \leq 0.01$) and divided ($p \leq 0.05$) into HRs and LRs VEHDMF but not STZDMF group as compared to the control VEHCTR rats, in particular in the LRs. The obtained results suggest that STZ alone (sAD model without DMF) and with the DMF treatment influence inflammatory response, as indicated by plasma CORT level, in old rats with different behavioral/stress reactivity

MEDIAL SEPTAL NMDA RECEPTOR INHIBITION AFFECTS PERIPHERAL BLOOD IMMUNE CELL DISTRIBUTION IN RATS DIFFERING IN THEIR LOCOMOTOR RESPONSE TO NOVELTY TEST. Chwiej, Monika 1, Podlacha, Magdalena 1, Laska , Ewa 1, Wrona, Danuta 1; 1Departament of Animal and Human Physiology, University of Gdansk, Gdansk, PA 80-309.

In the present study, an influence of the NMDA receptor antagonist – D-AP7 infusions into the medial septum (MS) on some immune parameters, including a number of peripheral blood leukocytes in rats differing in their locomotor response to novelty and stress susceptibility (high (HR) and low (LR) responders), was determined. Male Wistar rats (n=16), divided into the HRs and LRs (novelty test), were subjected to the D-AP7 (0.1 µg/rat; HR=5, LR=5) or saline (SAL) (0.5 µl/rat; HR=3, LR=3) injection via implanted into the MS cannulae. Blood samples were taken (200 µl) and total white blood cell number (WBC) and percentage of lymphocytes (LYM), monocytes (MONO) and eosinophils (EOS) was examined (Cell-Dyn 3700 analyzer). The D-AP7 injection significantly ($p \leq 0.001$) decreased the WBC number in the LRs (2410±640 cells) and percentage of MONO in the HRs ($p \leq 0.05$; 3.64±1.45%) in comparison with the SAL group (WBC: 6330±770 cells; MONO: 6.18±1.18%). There was a significantly decreased percentage of EOS ($p \leq 0.01$) in the HRs (1.85±0.51%) whereas it was increased in the LRs (2.82±0.70%, $p \leq 0.05$) within the D-AP7 group as compared to the controls (HRs: 3.18±0.22%; LRs: 1.58±0.33%). Moreover, a significant HRs vs. LRs difference with a higher WBC number ($p \leq 0.01$) in the HRs but no significant changes in the lymphocyte number after the D-AP7 injection, was noticed. The obtained results show

that the MS NMDA receptor inhibition differentially influence the distribution of the peripheral blood immune cells which could be attributed to the HRs vs. LRs differences and stress susceptibility.

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ACUTE EXPOSURE TO METHAMPHETAMINE ALTERS TLR9-MEDIATED CYTOKINE EXPRESSION IN MACROPHAGE. Ciborowski, P, Ph.D. 1, Burns, A, Ph.D. 1; 1University of Nebraska Medcial Center, Pharmacology and Experimental Neuroscience, Omaha, NE 68198.

Methamphetamine (Meth) is one of the most addictive and destructive illicit drugs. Besides acceleration of the central nervous system destruction, Meth has the ability to disrupt immune homeostasis and impact key subsets of leukocytes. Individuals addicted to Meth are also more susceptible to infections such as Methicillin Resistant Staphylococcus aureus (MRSA), cryptococcal infections, histoplasmosis, HIV-1 as well as sexually transmitted infections. Increased susceptibility to infectious diseases suggests that Meth impairs the immune system by altering its first line of defense - the innate immune system and its key player, macrophage. We profiled 84 cytokines and chemokines during 24h of Meth treated THP-1 cells, as a human macrophage model. Using up to 100uM of Meth and high throughput screening we found signaling targets of Meth. We showed that after a single exposure, the effect of Meth on macrophage cytokine production was rapid and time dependent and shifted the expression of cytokines to pro-inflammatory. The mRNA expression profile resulted in identification of 58 out of 84 differentially expressed mediators with more than two-fold up or downregulation in at least one time point. Meth elicited a strong (more than 10-fold) up-regulation of CXCL16 and CXCL2, moderate (5- to 10-fold) up-regulation of IL-7, CCL20, CXCL1, CCL24, and IL-8, and strong (more than 10-fold) down-regulation of CCL7 (MCP-3). Further we found that Meth dysregulates the MyD88-dependent Toll-like receptor 9 (TLR9) signaling pathway suppressing the expression of CCL7 and IFN-a.

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DUAL ACTIVATION OF ASTROCYTE TAAR1 AND TRP CHANNELS MITIGATES AUTOPHAGY DURING METH-ASSOCIATED HYPERTHERMIA IN THE CONTEXT OF HAND. Cisneros, IE, Ph.D. 1, Ghorpade, A, Ph.D. 1; 1Cell Biology and Immunology, University of North Texas Health Science Center, Fort Worth, TX 76107.

Methamphetamine (METH) abuse accelerates the severity and onset of HIV-associated neurocognitive disorders (HAND) and mediates molecular mechanisms including brain hyperthermia, astrocyte activation and downstream stress responses. METH targets astrocyte trace amine associated receptor (TAAR)1, previously implicated in thermoregulation; however, the molecular mechanisms remain elusive. Interestingly, TAAR1 signaling activates, regulates and sensitizes temperature receptive transient receptor potential (TRP) channels. TRP channels are ubiquitously expressed in cells throughout the periphery and CNS. Upon activation, TRP channels increase intracellular calcium concentrations ([Ca+2]i), and are critical in regulating body temperature during METH abuse. Likewise, METH-induced TAAR1 activation increases [Ca+2]i. Calcium imbalances are implicated in mitochondrial dysfunction, oxidative and ER stress thereby resulting in apoptosis and/or autophagy. Data shows aberrations in ER stress and autophagy markers in astrocytes challenged with METH, HIV-1 and associated hyperthermia.

We propose dual activation of astrocyte TAAR1/TRP channels via METH and associated hyperthermia, results in calcium dysregulation, mitochondrial dysfunction and autophagy. We will investigate the mechanistic roles of calcium signaling and mitochondrial damage leading to autophagy. Overall, this work identifies dual activation of astrocyte TAAR1/TRPs, synergistically induce molecular mechanisms regulating METH-associated transient hyperthermia-induced autophagy in the context of HAND.

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ELUCIDATING THE MECHANISMS OF HIV-ASSOCIATED P-SELECTIN GLYCOPROTEIN-1 DYSREGULATION. Connor, RP, MS 1, Jones, LD, MS 1, Singh, VB, Ph.D. 1, Thakar, J, Ph.D. 1, Maggirwar, SB, Ph.D. 1; 1Department of Microbiology and Immunology, University of Rochester Medical Center, Rochester, NY 14642.

With the advent of Highly Active Antiretroviral Therapy, HIV-infected individuals have a much increased life expectancy. However, these individuals are at increased risk of developing a wide range of complications, with monocyte tissue-infiltration underlying many of these, including HIV-Associated Neurocognitive Disorder. Thus, it was hypothesized that monocytes are altered during HIV infection so as to promote transmigration. P-Selectin Glycoprotein Ligand-1 (PSGL-1) is crucial for leukocyte transmigration, and flow-cytometric analyses revealed that PSGL-1 is significantly increased on monocytes from HIV-infected individuals. Further, it was found that the physiologically relevant mediators, sCD40L and glutamate, together induce PSGL-1 expression on monocytes isolated from healthy subjects, via transcriptional up-regulation. Additionally, in a mouse model of HIV infection, PSGL-1 and glutamate were found to be induced upon infection, in a CD40L dependent manner. To identify the mediators of this induction, a boolean network model of CD40L and glutamate signaling was developed, and this model was found to accurately predict known aspects of CD40L signaling. Further, it suggests that the transcription factor complex cMyc/MAX may be crucial for PSGL-1 induction. In support of this, microarray analyses predict cMyc/MAX transcription factor enrichment with HIV infection. Not only is this the first report of factors that regulate PSGL-1 transcription, it also highlights novel therapeutic targets to address pathologic neuro-inflammation during HIV infection.

THE KYNURENINE PATHWAY IN RATS RESISTANT TO ANTIDEPRESSANT EFFECT OF IMIPRAMINE. The Katarzyna Curzytek[1], Weronika Duda[1], Eimear Fagan[3], Thomas Connor[3], Piotr Gruca[2], Monika Leśkiewicz[1], Magdalena Regulska[1], Anna Kurek[1], Jan Detka[1], Barbara Korzeniak[1], Marta Kubera[1] [1]Department of Experimental Neuroendocrinology, [2]Laboratory of Behavioral Pharmacology Institute of Pharmacology, Polish Academy of Sciences, Smetna 12 street, 31-343 Krakow, Poland[3]School of Medicine & Trinity College Institute of Neuroscience, Trinity College, Dublin 2, Ireland

The major route of tryptophan metabolism, i.e. the kynurenine pathway is suspected to be related to the pathophysiology of depressive disorders and is responsible for approximately 99 % of the whole brain tryptophan metabolism *via* its degradation to kynurenine (KYN) catalyzed by indoleamine 2,3-dioxygenase (IDO). KYN is further converted by kynurenine aminotransferase (KAT) or kynurenine 3-monooxygenase (KMO). In former studies, we established that chronic mild stress (CMS) procedure decreased KAT II and increased IDO expression in the rat cortex.

The aim of the present study was to determine whether the administration of imipramine (IMI) to CMS rats can reverse these changes. We confirmed that the CMS procedure modeled one of the main symptoms of depression, i.e. anhedonia, and administration of IMI for 5 weeks resulted in a significant reduction in anhedonia in a majority of animals, although about 20% of animals did not respond to the IMI treatment. Chronic IMI administration to CMS rats decreased IDO and KMO expression and increased KAT II/KMO ratio in IMI responders in comparison to CMS rats. In IMI non-reactive rats, a significant increase in IDO expression and decrease in KAT II/KMO ratio in comparison with IMI responders was observed. The IDO activation reduced the availability of tryptophan to serotonin biosynthesis whereas the decrease in KAT II/KMO ratio was connected with increased production of neurotoxic 3-hydroxykynurenine by KMO and/or with decreased production of neuroprotective kynurenic acid by KAT. We hypothesize that the antidepressant activity of IMI partially depends on the modulation of brain KAT II/KMO ratio.

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ROLE OF HIV-1 TAT, COCAINE AND MIR-29B IN THE REGULATION OF EXCITATORY AMINO ACID TRANSPORTER, EAAT2 EXPRESSION IN ASTROCYTES. Datta, PK, Ph.D. 1, Deshmane, S, Ph.D. 1, Douthitt, ML, BS 1; 1Neuroscience, Lewis Katz School of Medicine at Temple University, Philadelphia, PA 19140.

HIV-1 Tat protein plays a critical role in the pathogenesis of HIV-1 associated neurocognitive disorders (HAND). Studies have also shown that the abuse of cocaine by HIV-1 infected individuals also contributes to the pathogenesis of HAND. In this study, we demonstrate that HIV-1 Tat alone and Tat in combination with cocaine induce the expression of miR-29b in primary human fetal brain astrocytes and microglia. Furthermore, extracellular vesicles (EVs) released from Tat alone and Tat plus cocaine treated microglia are enriched in miR-29b. Treatment of primary astrocytes and astrocytic cells U87 with EVs from treated microglia resulted in decreased expression of EAAT2 the most abundant glutamate transporter in astrocytes that plays a key role in regulating glutamate levels in the brain. We also show that EAAT2 is a target for miR-29b as evidenced by the interaction of miR-29b with the 3'-UTR of EAAT2 mRNA. Furthermore, we also observed that overexpression of miR-29b in U87 cells and primary astrocytes inhibited expression of EAAT2. These results demonstrate the role of microRNAs induced by HIV-1 Tat and cocaine in the post-transcriptional regulation of EAAT2 expression in astrocytes.

Supported by NIH/NIDA.

AUGMENTATION OF PULMONARY VASCULOPATHY AND HEMODYNAMICS IN HIV-TRANSGENIC RATS ON EXPOSURE TO COCAINE. Dhillon, ND, Ph.D. 1, Dalvi, P 1, Spikes, L 1, Julie, J 1, Gupta, V 2, Sharma, H 1, Gillcrist, M 1, Montes de Oca, J 1, O'Brien-Ladner, A 1; 1Internal Medicine, University of Kansas Medical Center, Kansas City, KS 661602Molecular & Integrative physiology, University of Kansas Medical Center, Kansas City, KS 66160.

IL-1BETA AUTOCRINE LOOP DIFFERENTIALLY REGULATES ASTROCYTE INFLAMMATORY RESPONSES IN HAND. Edara, VVC, MS 1, Ghorpade, A, Ph.D. 1; 1University of North Texas Health Science Center, Graduate School of Biomedical Sciences, Fort Worth, TX 76107.

HIV-1 infection of the central nervous system (CNS) impairs brain function and leads to HIV associated neurocognitive disorders (HAND). Astrocytes are the most abundant cell type in CNS and provide structural and metabolic support under homeostasis and in diseases including HAND. It is well established that IL-1beta is regulated in an autocrine fashion. Given our prior work on astrocyte inflammatory responses in HAND, we sought to investigate the role of IL-1beta autocrine loop in differential outcomes. Successfully knocking down new synthesis of IL-1beta by RNAi, changes in levels of CXCL-8, TNF-alpha, AEG-1, EAAT-2, TAAR-1 and TIMP-1 were measured in response to IL-1beta stimulation. As expected, RT2-PCR data confirmed that there was an increased mRNA expression of CXCL-8, TNF-alpha, TAAR-1 and TIMP-1 and no changes in EAAT-2 and AEG-1 levels. This suggests that IL-1beta autocrine loop likely plays a differential role in astrocyte inflammatory responses. Furthermore, we are particularly interested in differential response of healthy versus latently HIV-1-infected astrocytes that act as viral reservoir in CNS. Astrocytes were infected with the help of doubly fluorescent labelled pseudotyped HIV-1, and latently infected ones were sorted. Our long term goal is to delineate the specific role of IL-1beta autocrine loop in differential regulation of inflammatory responses in latently infected astrocytes. These studies are highly significant to address CNS reservoir issues in post ART HAND.

HSV-1 REPLICATION KINETICS AND IMMUNE RESPONSE IN THE LIP SCARIFICATION MODEL OF INFECTION. Egan, KP, BS 1, Turner, P, BS 1, Wigdahl, B, Ph.D. 1, Jennings, SR, Ph.D. 1; 1Department of Microbiology and Immunology, Institute for Molecular Medicine and Infectious Disease, Drexel University College of Medicine, Philadelphia, PA 19102.

Herpes simplex virus type 1 (HSV-1) is a human pathogen that replicates in epithelial cells of mucosal surfaces before establishing a lifelong latent infection within the trigeminal ganglion (TG). The immune system is critical for establishing and maintaining latency. HSV-1 disease occurs with a range of severities and can include corneal scarring and blindness to mild recurrent lesions following infection of the eye and lip, respectively. There is an established ocular infection model in the laboratory mouse which reproduces primary infection and latency observed in humans. However the majority of primary human infections occur within the lip and latency is established within a different branch of the TG from the ocular model. We set out to define the kinetics of HSV-1 replication and CD8+ T-cell response in the lip scarification model. The lower lip of three month old mice were scarified and 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 titers of virus were found in the lip at early time points that resolved after 8 days of exposure. Lip pathology peaked 5 days post-infection and resolved 15 days post-infection. CD8+ T cells were retained in the trigeminal ganglion 30 days post-infection. These results show that the lip scarification model can reproduce human infection with characteristic pathology, resolution, and retention of CD8+ T cells in the TG.

THE ROLE OF MICRORNAS FROM MICROGLIA-DERIVED EXTRACELLULAR VESICLES DURING METHAMPHETAMINE ABUSE. Fernandes, N, MS 1, Potula, R, Ph.D. 1; 11Department of Pathology and Laboratory Medicine, Lewis Katz School of Medicine, Temple University, Philadelphia, PA 19140.

Extracellular vesicles (EVs), once considered cellular waste, are emerging as mediators of intercellular communication. EVs express cell-specific transmembrane markers and several protein and nucleic acids within their lumen, which alter the function of target cells. However, the role of EVs within the CNS remains unclear, particularly within the context of neuroinflammation. Microglia are the primary immune cells in the CNS and mediate inflammatory responses against danger signals. Evidence suggests that microglial purinergic receptors, particularly P2X7R, may play a role in mediating the EV generation through inflammasomes, contributing to neuroinflammation. Methamphetamine (METH) is an addictive psychostimulant that causes sustained microglial activation with chronic abuse. Microglial response to METH appears to precede neuronal loss and presents a critical point of intervention in understanding, preventing, and treating neuroinflammation. However, microglia-derived EVs have not been characterized in humans. To elucidate the role of microglia-derived EVs during METH-induced microgliosis, primary human microglia were stimulated with METH. EVs were isolated from supernatants using ultracentrifugation followed by incubation in ExoQuick. Human microglia-derived EVs were characterized via western blot analysis using markers of EVs. Transcriptome profiling of the EV contents revealed alterations in several miRNAs implicated in neuroinflammation. These findings provide, for the first time, a novel way in which microglia contribute to neuroinflammation during substance abuse.

Supported by NIH.

NEUROPROTECTIVE EFFECTS OF FK506 IN A MODEL OF HIV1-GP120 NEUROTOXICITY. Fields, JA, Ph.D. 1, Overk, C, Ph.D. 2, Adame, A, BS 2, Florio, J, BS 2, Mante, M, BS 2, Pineda, A, BS 2, Desplats, P, Ph.D. 2, Rockenstein, E, BS 2, Masliah, E, MD 2; 1Department of Pathology, University of California, San Diego, La Jolla, CA 920932Department of Neurosciences, University of California, San Diego, La Jolla, CA 92093.

HIV-associated neurocognitive disorders (HAND) continue to be a common morbidity in patients HIV. It has been shown that HIV proteins (eg: gp120) released from infected microglial/macrophage cells can cause neuronal damage by triggering inflammation and oxidative stress, activating aberrant kinase pathways, and by disrupting mitochondrial function and biogenesis. Previous studies have shown that FK506, an immunophilin ligand that modulates mitochondrial function and binds calcineurin, is capable of rescuing the neurodegenerative pathology in models of Parkinson's disease, Alzheimer's disease and Huntington's disease. In this context, the main objective of this study was to evaluate if FK506 could rescue the neuronal degeneration and mitochondrial alterations in a transgenic (tg) animal model of HIV1-gp120 neurotoxicity. For this purpose, GFAP-gp120 tg mice were treated with FK506 and analyzed for neuropathology, behavior, mitochondrial markers and calcium flux by 2 photon microscopy. We found that FK506 reduced the neuronal cell loss and neuro-inflammation in the gp120 tg mice. Moreover, while vehicle-treated gp120 tg mice displayed enlarged mitochondria and alterations in biogenesis, FK506 rescued the morphological mitochondrial alterations and increased levels of OPA1 and MFN1. By 2-photon microscopy calcium levels were not affected in the gp120 tg mice and no effects of FK506 were detected. However, at a functional level, FK506 ameliorated the gp120 tg mice hyperactivity in the open field. Together, these results suggest that FK506 may be neuroprotective in HAND.

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REGULATION OF HEMATOPOIETIC STEM CELL (HSC) DEVELOPMENT: ROLE OF GLUCOCORTICOID INDUCED LEUCINE ZIPPER (GILZ). Frammartino, T, Ph.D. 1, Cimino, M, Ph.D. 1, Sorcini, D, Ph.D. 1, Biagioli, M, Ph.D. 1, Bruscoli, S, Ph.D. 1, Bereshchenko, O, Ph.D. 1, Riccardi, C, MD, Ph.D. 1; 1Department of Medicine, Section of Pharmacology, University of Perugia, Perugia, ID 06100.

The immune/inflammatory cell system derives from the differentiation of hemopoietic stem cells (HSC). Glucocorticoids (GC) are important anti-inflammatory drugs used in therapy of many autoimmune/inflammatory diseases including neurodegenerative diseases. GC interfere with stem (HSC) and progenitor (HPC) cells development. Endogenous glucocorticoid hormones (GC) influence the proliferation and rhythmic egress of HSC from bone marrow via regulation of CXCL12-CXCR4 axis. Most of GC effects are receptor (GR) mediated and relate to regulation of gene transcription. GILZ (Glucocorticoid-Induced Leucine Zipper) is a gene rapidly induced by GC that mediates some of its effects, including regulation of cell growth and differentiation. gilz mRNA is expressed at higher levels in long term (LT-HSC), short-term HSCs and lymphoid-myeloid primed (LMPP), compared to myeloid progenitor cells. We have addressed the role of GILZ on HSC and progenitor cell homeostasis. Our results indicate that lack of GILZ causes a significant decrease of LT-HSC and a parallel increase of LMPP. Cell cycle analysis of GILZ deficient, freshly isolated bone marrow show a significantly increased cell cycling, as demonstrated by ki67 staining, that results in decrease of the self-maintenance capability of HSC. Consequently, donor bone marrow cells of GILZ lacking mice are less efficient in self-maintaining in transplanted recipients. In conclusion, results indicate that GILZ plays a role in regulation of HSC and HPC activity and suggest GILZ is a mediator of GC effects on hemopoietic system.

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EXPRESSION OF DOPAMINERGIC RECEPTORS ON HUMAN MONOCYTES AND PERIPHERAL BLOOD DENDRITIC CELLS. Gaiazzi, MG, MD 1, Rasini, ER, BS 1, Marino, FM, BS 1, Zaffaroni, MZ, MD 2, Cosentino, MC, MD, Ph.D. 1; 1Center of Research in Medical Pharmacology, University of Insubria, Varese, 211002Multiple Sclerosis Center, S. Antonio Abate Hospital, Gallarate, 21013.

The neurotransmitter dopamine exerts its effects on five dopaminergic receptors (DR) that are expressed also on immune cells. The presence of DR has been reported in human immature monocyte-derived dendritic cells (DC) as well as on human CD14+CD16+ monocyte (MO). Few information however exist so far on the role of DR in DC and MO. The aim of our study was to assess DR expression in human MO, circulating DC (bDC) and their respective subsets in healthy subjects. Peripheral venous blood samples were collected, and phenotyping of DR on MO and bDC was performed by flow cytometric analysis using a two-step protocol which allowed the identification of all the five DR on classical (Cl: CD14+CD16-) and non-classical (NCl: CD14dimCD16++) MO, and on plasmacytoid DC (pDC) and myeloid DC (mDC). Preliminary results obtained so far showed that staining for DR on bDC as well as pDC or mDC provided negligible expression for all DR; on the contrary, all five DR are expressed in circulating MO, with higher frequencies in the Cl subtypes (DR D1 [%,mean±SD]: 53.4±37.0% in CI vs 97.8±0.9% in NCl; D5: 51.4±34.6% vs 98.5±0.3%; D2: 64.7±30.1% vs 95.7±2.4%; D3: 38.8±37.5% vs

97.3±0.8%; D4: 45.4±29.6% vs 96.9±1.0%; all: n = 4, P<0.05 vs NCl). Results suggest that DR are not expressed on bDC, while they are highly expressed on MO, however to a different extent on the various subsets. NCl MO increase in humans during inflammation and systemic infection, thus the DR highly expressed on these cells might represent a potential target to modulate their functions in health and disease.

THE NEONATAL ANTI-VIRAL RESPONSE IN THE CNS IS ASSOCIATED WITH IMMUNE CELL INFILTRATION AND CYTOKINE PRODUCTION, BUT FAILS TO CONTROL MEASLES VIRAL SPREAD . Ganesan, PG, BS 1, Bohn, LB, BS 1, O'Donnell , LOD, Ph.D. 1; 1Duquesne University, Mylan School of Pharmacy, Pittsburgh, PA 15208.

Viral infections in the central nervous system (CNS) are associated with devastating neurological consequences, particularly in newborns. To study viral CNS infections in neonates, we use a transgenic mouse model of neuronally-restricted measles virus (MV) infection (NSE-CD46 mice), where the human isoform of CD46, a MV receptor, is expressed under the control of the neuron-specific enolase promoter. Adult CD46+ mice survive infection and clear MV in an interferon gamma (IFNg)- and T cell-dependent manner. In contrast, neonatal CD46+ mice succumb to the infection despite T cell infiltration into the brain parenchyma. Neonatal mice lacking IFNg succumb more rapidly than wildtype pups despite higher T cell infiltration, equivalent natural killer cell infiltration, and microglial activation. Quantitative RT-PCR analysis demonstrated upregulation of pro-inflammatory cytokines and chemokines such as IFNg, IL-1β, and CXCL10, as well as the anti-inflammatory interleukin 1 receptor antagonist in infected adults and neonates. IL-12β, IL-23, CCL2, and TNF were significantly upregulated in infected neonates only. These results suggest the neonates are capable of cytokine production with a Th1-like profile within the CNS, but that the cytokine milieu is ineffective at controlling MV spread. Current experiments aim to inhibit inflammatory mediators that are expressed in an age-dependent manner during infection to determine the role of individual cytokines/chemokines in neonatal neuropathology. This study will enable us to identify therapeutic targets in neonatal viral infections.

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SEX MODERATES NEUROCOGNITION IN A PRECLINICAL MODEL OF HAND. Gassmann, M.R., BS 1, McLaurin, K.A., BS 1, Booze, R.M., Ph.D. 1, Mactutus, C.F., Ph.D. 1; 1Department of Psychology, University of South Carolina, Columbia, SC 29208.

Direct comparison of rates of neurocognitive impairment for men vs. women in HAND are needed to evaluate possible sex differences; the same need exists in preclinical models of HAND. Using a longitudinal study, and a population approach (ns=28-32), we assessed the neurocognitive performance of male and female Fischer-344N HIV-1 transgenic rats (Tg) and non-transgenic controls, beginning at 60 days of age, using a signal detection task as an index of sustained attention. During task acquisition (70% accuracy for 5-7 days) there was no compelling evidence for any impaired learning by the HIV-1 Tg rats, albeit there was a robust sex difference with 50% of the males reaching criteria by 36 days vs. 62 days for females. Further, at asymptotic performance there was no difference between Tg and controls on reinforced (hits and correct rejections) and non-reinforced (misses and false alarms) trials, however,

males made significantly more responses than females on both reinforced (72% & 79%) and non-reinforced (32% & 44%) trials. Sex moderated the impact of the HIV-1 transgene on performance of the signal detection task: the signal duration at which males failed to distinguish between hits and misses shifted 125 msec as a function of the HIV-1 transgene, whereas the shift for females was 250 msec. In summary, sex differences in performance of the signal detection task were apparent in shaping (data not discussed), acquisition, and asymptotic performance; most critically, sex moderated the influence of the HIV-1 transgene on detectability of signals as a function of their duration.

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LOSARTAN AND B-SECRETASE INHIBITOR REGULATE THE AMYLOID PRECURSOR PROTEIN CLEAVAGE INDUCED BY ANGIOTENSIN II IN HUMAN NEURONAL CELLS. Gerena, Y, Ph.D. 1, Delgado, A, BS 2, Velez, JG, BS 2, Ayala, G, BS 2, De Jesus, E, BS 1, Marrero , L, BS 1, Flores, M, BS 1, Wojna, V, MD 3; 1Pharmacology Department, University of Puerto Rico, Medical Sciences Campus, San Juan, PR 009362Rio Piedras Campus, University of Puerto Rico, Rio Piedras, PR 009313Neurology Division, University of Puerto Rico, Medical Sciences Campus, San Juan, PR 00936.

Angiotensin II (AngII) receptor blockers (ARBs) are associated with enhancement of cognitive function in Alzheimer's disease and other neurodegenerative disorders. However, little is known about the mechanisms responsible for the ARBs benefits in these disorders. Here, we investigated if AngII influences the membrane amyloid precursor protein (mAPP) levels and β-secretase1 (BACE1) activity in human neuronal cells and then analyzed its effects in the presence of Losartan (AT1-receptor blocker), PD123177 (AT2-receptor blocker), and a BACE1 inhibitor. Human neuronal cells (SH-SY5Y; 5x10E6cells) were cultured in the presence of AngII (600nM) for 24hr and the mAPP levels and BACE1 activity were analyzed using an APP antibody for flow cytometry and a fluorescent BACE1 substrate, respectively. The mAPP levels and BACE1 activity were also measured in cells exposed to AngII plus Losartan (1uM), AngII plus PD123177 (1uM), AngII plus BACE1 inhibitor (1uM), or AngII plus a combination of the drugs. Our results indicated that: (1) Neuronal mAPP levels significantly decreased (p=0.04) and BACE1 activity increased (p=0.01) after Ang II incubation; (2) Both Losartan and BACE1 inhibitor antagonized the effects of AngII; (3) No significant differences were observed with PD123177; (4) Treatment with Losartan plus BACE1 inhibitor did not produce a synergistic antagonism of AngII effects on mAPP levels. Our findings support that AngII has a role in the mAPP cleavage through BACE1 activation. This study may help to clarify the benefits of ARBs and BACE1 inhibitors in neurodegenerative disorders.

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REGULATION OF MITOCHONDRIAL PROTEIN EXPRESSION IN THE HIPPOCAMPUS BY ACUTE PERIPHERAL LIPOPOLYSACCHARIDE ADMINISTRATION- STUDY IN AN ANIMAL MODEL OF DEPRESSION. Glombik, K, Ph.D. 1, Stachowicz, A, Ph.D. 2, Olszanecki, R, MD, Ph.D. 2, Trojan, E, MS 1, Slusarczyk, J, MS 1, Basta-Kaim, A, Ph.D. 1; 1Department Of Experimental Neuroendocrinology, Institute of Pharmacology Polish Academy of Sciences, Cracow, 31-3432Chair of Pharmacology, Jagiellonian University Medical College, Cracow, 31-531.

Current research indicates that stress during the perinatal period increases the sensitivity of brain mitochondria to the exposure to adverse factors later in life e.g. inflammation. Our goal was to determine the influence of LPS, a bacterial endotoxin, on the mitoproteome of the hippocampus of adult male rats after prenatal stress procedure. Pregnant Sprague-Dawley rats were subjected to stress session from 14th day of pregnancy until delivery. At 3 months of age prenatally stressed male rats after behavioral verification were divided into two groups: prenatally stressed and prenatally stressed +LPS. Lipopolysaccharyde (LPS; 250 ug/kg) was injected once i.p. and 4 hours later animals were killed by rapid decapitation and the hippocampi were dissected out. 2DE-LC-MS/MS methodology was applied to investigate the changes of expression in mitochondrial proteins. We identified 18 differentially expressed mitochondrial proteins in the hippocampus of prenatally stressed rats treated with LPS compared to prenatally stressed rats. Proteomic analysis reavaled that disturbances in Krebs cycle in the brains of prenatally stressed rats observed in our previous studies have been intensified after LPS treatment in the hippocampus. We observed dramatically decreased expression of pyruvate dehydrogenase E1 complex subunit alpha (below the level of detection) and isocitrate dehydrogenase (fold change -18,22) . Further studies should determine the precise mechanisms involved in the multi-factorial interaction between inflammatory activation and hippocampal mitochondrial function.

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EFFECT OF CAFFEINE GIVEN CHRONICALLY WITH MDMA OR METHAMPHETAMINE IN A ‘BINGE’ MODE OF TREATMENT ON DA AND 5-HT EXTRACELLULAR LEVEL IN THE MOUSE STRIATUM. Górska, A.M., MS 1, Gołembiowska, K., Ph.D. 1; 1Department of Pharmacology, Institute of Pharmacology, Polish Academy of Sciences, Kraków, 31-343.

3,4-methylenedioxymethamphetamine (MDMA), psychostimulant with addictive potential is one of the most popular ‘club drug’ especially in a group of young people. In recent years MDMA users often ingest MDMA in a short time interval concomitantly with caffeine (CAF), as a ‘energy drink’ or coffee, to gain stronger stimulatory effect or alleviate decrease in mood and fatigue during abstinent period. It is not known if combination of MDMA and methamphetamine (METH) with CAF is beneficial or has adverse impact on the central nervous system. The aim of our research was to investigate the effect of CAF on repeated doses of MDMA and METH on DA and 5-HT release in the mouse striatum using microdialysis in freely moving animals. Extracellular level of DA and 5-HT was assayed by HPLC with coulochemical detection. MDMA (4 x 10 mg/kg/2 days for three weeks) and METH (3 x 5 mg/kg/2 days for three weeks) markedly increased release of DA and 5-HT. CAF (2 x 5 mg/kg for three weeks) given together with MDMA and METH lowered extracellular level of DA and 5-HT. Our findings indicate potentiation by CAF possible depleting effect of MDMA and METH on monoamine terminals.

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A MOLECULAR GENETIC ASSOCIATION OF BLOOD BRAIN BARRIER DYSFUNCTION IN SCHIZOPHRENIA. Greene, C, BS 1, Humphries, MM, Ph.D. 1, Kealy, J, Ph.D. 1, Murphy, K, MD 2, Campbell, M, Ph.D. 1;

1Smurfit Institute of Genetics, Trinity College Dublin, Dublin, -2Department of Psychiatry, Beaumont Hospital, Dublin, -.

Schizophrenia is a debilitating psychosocial disorder characterized by abnormal social behavior, auditory hallucinations and a failure to recognize what is real. While much research has focused on susceptible genetic loci and dysfunctional dopamine neurotransmission as hallmarks of the condition, we sought to assess the contribution of the microvasculature to its onset. A key component of the cerebral vasculature is the blood brain barrier (BBB), a highly selective barrier system that maintains homeostasis of ions within the CNS for optimal synaptic signalling and restricts movement of certain molecules from blood to brain and vice-versa. Using a molecular genetics based approach, we show that the key tight junction component, claudin-5, is associated with schizophrenia in a cohort of patients at high risk of developing schizophrenia. In the chromosomal abnormality, 22q11 deletion syndrome (22q11DS), individuals have a 1.5-3 Mb deletion on chromosome 22q11.21, the region where claudin-5 is located. A variant that involves a G to C base change in the 3' untranslated region of the claudin-5 gene is significantly associated with schizophrenia in a population of individuals with 22q11DS. Functional analyses of the variant allele conferred a dysfunctional BBB phenotype with reduced claudin-5 protein expression in vitro. Furthermore, in an in vivo study with a doxycycline inducible claudin-5 "knockdown" mouse, reduced claudin-5 expression resulted in a decreased acoustic pre-pulse inhibition, a classical and well conserved schizophrenia phenotype in rodents and human subjects.

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SLPI IS INVOLVED IN THE INDUCTION OF NGF IN THE PSORIATIC SKIN . Grygier, B., Ph.D. 1, Majchrzak-Górecka, M., MS 1, Majewski, P., Ph.D. 1, Cichy, J., Ph.D. 1; 1Faculty of Biochemistry, Biophysics and Biotechnology, Jagiellonian University, Krakow, 30-387.

Psoriasis is suggested to be a chronic skin disease caused by abnormal neuro-immune processes. The undesirable pathways are mediated by mast cells and their mediators, which include proteases and nerve growth factor (NGF). Proteases inhibitors such as the secretory leukocyte protease inhibitor (SLPI) may be useful tool for management of the proper interaction between the both systems, although the impact of SLPI on NGF synthesis and function remains unknown. In the present study we aimed to investigate whether the elevated level of NGF in psoriatic skin depends on SLPI. For this purpose we used an experimental model of psoriasis based on Aldara cream (5% imiquimod, TLR7 agonist) treatment. In the first three days of Aldara application the epidermal thickness was significantly higher in SLPI-/- mice, indicating an intensive keratinocyte proliferation. In turn, after three days the trend was reversed: the epidermis and the entire skin thickness were significantly greater in SLPI+/+ mice. Additionally, skin morphological changes corresponded well with alterations in skin NGF mRNA level. There was an up-regulation of NGF in SLPI-/- mice in the first two days, but in the third day the NGF drastically decreased. In SLPI+/+ mice NGF mRNA increased much later, not until the fourth day. These findings suggest that the kinetics of NGF induction in psoriatic skin depends on the presence of SLPI. Therefore, further characterization of the relation between SLPI and NGF in inflammatory condition may represent an interesting direction in studying the neural background of immune response.

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EPIGENETIC DOWNREGULATION OF MICRORNA-124 PROMOTES COCAINE-MEDIATED MICROGLIAL ACTIVATION: IMPLICATION FOR DRUG ADDICTION . Guo, M, Ph.D. 1, Periyasamy, P, Ph.D. 1, Callen, S 1, Buch, S, Ph.D. 1; 1Department of Pharmacology and Experimental Neuroscience, College of Medicine, Omaha, NE 68198.

Emerging evidence has demonstrated that altered cross-talking between glial cells and neurons contributes to drug addiction. Cocaine has been shown to potentiate the expression of neuroinflammation-related genes in multiple model systems. Recently cocaine was demonstrated to interact with the toll-like receptor 4 (TLR4) leading to enhanced neuroimmune signaling and ensuing addiction-related behavioral changes. MicroRNAs have been shown a close association with drug addiction. Here we showed that miR-124 is involved in cocaine-mediated upregulation of TLR4 signaling. Our findings demonstrated that cocaine time- and dose- dependently attenuated miR-124 levels in both BV2 and rat primary microglia. Furthermore, results from in vivo studies validated the cocaine-mediated down-regulation of miR-124 expression. Cocaine-mediated downregulation of miR-124 involved epigenetic DAN methylation at the pri-miR-124 promoter. Krupple-like factor 4 (KLF4) was identified as a novel substrate modulated by miR-124. Besides KLF4, miR-124 also modulated the expression of several other proteins including TLR4, MyD88, TRAF6 and IRAK1. Overexpression of miR-124 in microglia blocked cocaine-mediated activation both in vitro and in vivo. In summary, our findings demonstrate a yet unexplored mechanism for cocaine-mediated activation of microglia via down-regulation of miR-124 (through hypermethylation), leading ultimately to increased TLR4 activation. MiR-124 could be considered as a potential target to ameliorate cocaine-mediated neuroinflammation and possibly addiction.

Supported by NIDA.

TWO NEW POTENTIAL BLOOD-BRAIN BARRIER PROTECTIVE AGENTS: EXPERIMENTS ON RAT BRAIN ENDOTHELIAL CELLS. Harazin, A, MS 1, Bocsik, A, MS 1, Vecsernyes, M, Ph.D. 2, Matyus, P, Ph.D. 3, Deli, MA, MD, Ph.D. 1; 1Biological Research Centre, Institute of Biophysics, Szeged, 67262University of Debrecen, Institute of Pharmaceutical Technology, Debrecen, 40323Semmelweis University, Department of Organic Chemistry, Budapest, 1092.

Protection of brain endothelial cells forming the functional blood-brain barrier (BBB) emerges as new therapeutic target. There is overwhelming evidence that brain capillary endothelial cells are damaged in pathologies of the nervous system and in systemic inflammations. Cytokines like tumor necrosis factor α (TNFα) and interleukin-1β (IL-1β) are inducers of both neuronal and peripheral inflammations. Two molecules were selected with proven anti-inflammatory effects: α-melanocyte stimulating hormone (αMSH), a neuropeptide, and SZV-1287, a newly synthesized inhibitor of vascular adhesion protein-1 (VAP-1). Our aim was to test the effects of these two compounds on primary brain endothelial cells and elucidate potential protective effects. Cellular damage in rat brain endothelial cells was induced by a combination of TNFα and IL-1β, and by L-ornithine, a cationic amino acid inducing acute pancreatitis in rats. Viability of brain endothelial cells was measured by real-time cell microelectric sensing (RTCA, ACEA) and verified by 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) assay. Integrity of brain endothelial cell layers was tested by measurements of electric resistance and

permeability. αMSH is not toxic to rat brain endothelial cells and has a protective effect on cell viability in low (0.001 nM) concentration against cytokine damage. SZV-1287 (1 μM) is also non-toxic to the isolated cells and can prevent the damage of high concentration (20 mM) of L-ornithine. Both compounds protected barrier integrity against damaging effects on our BBB model.

SELECTIVE GENERATION OF HUMAN DOPAMINERGIC PRECURSORS BY DIRECT LINEAGE CONVERSION. He, M, MS 1, Tian, C, Ph.D. 2, Li, Y, MS 2, Zhang, H, MD 2, Huang, Y, MD, Ph.D. 2, Zheng, J, MD 2; 1Medical Sciences Interdepartmental Area, University of Nebraska Medical Center, Omaha, NE 681052Department of Pharmacology and Experimental Neuroscience, University of Nebraska Medical Center, Omaha, NE 68105.

Transplantation of dopaminergic precursors (DPs) is a promising therapeutic strategy of Parkinson's disease (PD). However, limited cell source for dopaminergic precursors has become a major obstacle for transplantation therapy. Recently, we have demonstrated that mouse fibroblasts can be reprogrammed into induced dopaminergic precursors (iDPs) through ectopic expression of transcription factors Brn2, Sox2 and Foxa. These mouse iDPs selectively differentiated into dopaminergic (DA) neurons and showed no signs of tumor formation. In the current study, we hypothesize that similar strategy can be applied to generate human iDPs for future cell therapy of PD. We overexpressed transcription factors Brn2, Sox2 and Foxa2 in human fibroblasts and observed formation of neurospheres in the cultures. Subsequent characterization of the precursor colonies confirmed the generation of iDPs. These iDPs were capable of self-renewal, proliferate, and differentiation. The iDPs also expressed neural progenitor marker genes such as Nestin, Pax6 and ventral mesencephalon related genes such as Lmx1a, Corin and Otx2. More importantly, the iDPs expressed midbrain neural progenitor marker gene DCX, Corin and neural progenitor marker gene Nestin by immunostaining. Together, these results suggest that human iDPs can be generated by direct reprogramming of fibroblasts. These human iDPs may serve as a safe and effective cell source for transplantation treatment of PD.

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TETRAHYDROCANNABINOL (THC) SUPPRESSES CPG OLIGODEOXYNUCLEOTIDE (CPG)-MEDIATED ACTIVATION OF HUMAN PLASMACYTOID DENDRITIC CELLS (PDC). Henriquez, J. E., MS 1, Rizzo, M. D., BS 1, Schultz, M., BS 1, Crawford, R., BS 1, Kaminski, N., Ph.D. 1; 1Institute of Integrative Toxicology, Michigan State University, East Lansing, MI 48824.

Plasmacytoid dendritic cells (pDC) are key regulators of anti-viral immunity and bridge the innate and adaptive immune response by secreting IFNa and stimulating T cell clonal expansion. Tetrahydrocannabinol (THC), the major psychoactive component of medical marijuana, is a well-characterized immunosuppressant. The objective of this study was to determine if THC impairs pDC function by investigating IFNa secretion, a key early response, and CD83/CD86 expression, which are markers of DC maturation. We found that CpG induced IFNa secretion by pDC was suppressed in a concentration-dependent manner by THC but not by the cannabinoid receptor 1/2 low affinity agonist, cannabidiol. This observation was confirmed by measuring the IFNa secretion and IFNa mRNA expression in isolated pDC. CD83 was suppressed by THC at 6 and 24 hr post CpG stimulation compared

to control and CD86 was suppressed by THC at 24 hr post stimulation. To elucidate potential suppressive mechanisms of IFNa secretion and pDC maturation, we investigated Interferon response factor-7 (IRF-7), key-regulator of IFNa, and EAT-2, a SLAM adaptor protein that plays a crucial role in cytokine production and co-stimulatory molecule expression. THC mediated suppression of IRF-7 and EAT-2 occurred at 3 and 24 hr post stimulation, respectively. These data indicate THC markedly suppresses the pDC antiviral response, which may have consequences on T cell proliferation. These findings have potentially serious implications for immune compromised patients (i.e. HIV and Cancer patients) whom are prescribed therapeutic cannabinoids.

Supported by NIH RO1-DA007908.

METHADONE PROMOTES HIV-1 INFECTION OF MACROPHAGE BY INHIBITING THE EXPRESSION OF ANTIVIRAL GENES. Hou, Wei, MD, Ph.D. 1, Wu, Di-di, BS 1, Wang, Hui, BS 1, Li, Li, Ph.D. 1, Wang, Xiao-kun, Ph.D. 1, Xie, Lin-lin, Ph.D. 1, Luo, Fan, MD, Ph.D. 1; 1State Key Laboratory of Virology/Institute of Medical Virology/Hubei Province Key Laboratory of Allergy and Immunology, School of Basic Medical Sciences, Wuhan University, Wuhan, 430071.

Epidemiologic and experimental preclinical medicine studies support that opioid abusers have a higher risk of acquisition of diverse pathogens and exacerbating the severity of disease. An important reason is that their immune system can be damaged by illicit drugs (methamphetamine, heroin, morphine etc.). Methadone is a u-opioid receptor agonist as same as morphine. Compared with morphine, methadone is characterized by a long action time, less tolerance and low drug dependence, so it be served as a drug for the treatment of opiate addiction. However, we have known little about the impact of methadone on viral infections in host immune. Here we show that methadone enhanced the infection of HIV in macrophages by suppressing the expression of multiple factors in innate immunity. Methadone not only inhibited the expression of IFN-β and IFN-λ2, but also down-regulated the IFN-inducible anti-HIV genes expression, including APOBEC3F/G and MxB. In addition, methadone-treated macrophages expressed lower levels of microRNAs (miRNA-28, 125b, 150, and 155), which these microRNAs play an important role in limiting HIV infection. Our findings indicated that methadone could influence the innate immune response as well as other opioids, which provide a significant reference for methadone maintenance treatment.

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HYPERACTIVITY IS ALLEVIATED BY COMBINED CHRONIC BLOCKADE OF L-TYPE CA2+ CHANNELS AND NMDA RECEPTORS. Hu, X-T, MD, Ph.D. 1, Khodr, CE, Ph.D. 1, Chen, L, Ph.D. 1, Dave, S, Ph.D. 1, Al-Harthi, L, Ph.D. 2; 1Department of Pharmacology, Rush University Medical Center, Chicago, IL 606122Department of Immunology and Microbiology, Rush University Medical Center, Chicago, IL 60612.

HIV-1 infection induces neurological and neuropsychiatric deficits associated with dysregulation of the medial prefrontal cortex (mPFC) and other vulnerable brain regions. Although over-activation of NMDA receptors (NMDAR) is well described in the HIV infected brain, blocking NMDAR was not

beneficial in reversing HIV-induced neuronal damage in clinical trials. Here, we evaluated the impact of HIV in the mPFC and the therapeutic potential of targeting over-active voltage-gated L-type Ca2+ channels (L-channel) and NMDAR in HIV-1 transgenic (Tg) rats using whole-cell patch-clamp recording. Neurons from HIV-1 Tg rats showed reduced rheobase, spike amplitude and inwardly-rectifying K+ influx, increased firing number, and a trend of aberrant firing compared to those from non-Tg rats. This hyper-excitation was associated with enhanced Ca2+ influx (independent of NMDAR) that was eliminated by acute L-channel blockade. Combined, but not individual, chronic blockade of over-active L-channels and NMDARs abolished HIV effects on spiking, aberrant firing and Ca2+ potential half-amplitude duration, though not on reduced K+ influx. Our findings demonstrate that HIV alters membrane excitability and L-channel-mediated Ca2+ influx in mPFC neurons. This renders these neurons more susceptible and vulnerable to excitatory stimuli, and could contribute to HIV-associated neuropathogenesis. Combined targeting of over-active L-channels/NMDARs alleviates this HIV-induced dysfunction, emphasizing a potential novel therapeutic strategy that may effectively decrease HIV-induced Ca2+ dysregulation.

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SILICA NANOPARTICLES DE-ACIDIFY ENDOLYSOSOMES AND INCREASE AMYLOIDOGENESIS IN PRIMARY CULTURED NEURONS . Hui, L., MD, Ph.D. 1, Ye, Y., Ph.D. 1, Wollenzien, H. 1, Lakpa, L 1, Chen, X.S., MD, Ph.D. 1, Geiger, J.D., Ph.D. 1; 1Department of Basic Sciences, University of North Dakota, School of Medicine & Health Science, , grand forks, ND 58203.

Silica nanomaterials (SiNPs) are increasingly being used as carrier molecules in consumer products and in biomedicine. However, SiNPs may cause adverse effects including enhanced amyloidogenesis by promoting beta-amyloid (Aβ) fibrillation and Alzheimer's disease (AD) like pathology. Therefore, the extent to which SiNPs influence Aβ generation and the underlying mechanisms by which this occurs deserves further investigation. In this study, we found that following endocytosis of SiNPs (50 nM) into primary cultured hippocampal neurons, Aβ40 secretion was significantly increased and that this increase was accompanied by elevated protein expressions of N-APP and BACE-1. In addition, we found that SiNPs, caused rapid deacidification of neuronal endolysosome pH and that this increase in pH persisted for at least 24 hours after SiNP application. Furthermore, application of the lysosomotrophic agent glycyl-L-phenylalanine 2-naphthylamide induced lysosomal calcium release and this release was reduced by SiNPs. Moreover, two agents that cause endolysosome acidification, MLSA-1 (TRPML agonist) or CGS21680 (adenosine A2a receptor agonist), significantly decreased SiNP-induced endolysosome pH and Aβ40 generation. Collectively, our findings suggest that SiNP-induced deacidification of endolysosomes might be mechanistically linked to increased amyloidogenesis and such findings provide a cautionary note about using certain NPs because of potential negative effects on neurons.

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DIFFICULT NEUROLOGICAL COMPLICATION IN HIV-INFECTED YOUNG WOMAN. Ianache, I.C., MS 1, Oprea, A.C., MD, Ph.D. 1; 1"Victor Babes" Clinical Hospital for Infectious and Tropical Diseaseases, HIV/AIDS Department, Bucharest, 030303.

Background: Immune reconstitution inflammatory syndrome (IRIS) may represent a challenge for the management of HIV-infected patients. Case description: We present a 26 year-old woman, diagnosed with HIV infection, admitted in our hospital with mild depression and anxiety. Lab screens showed anemia, severe immunosuppression (CD4 cell count 63 cells/mm3) and high plasma HIV-RNA (6.25 log10 copies/mL). Antiretroviral treatment was initiated with 3TC/TDF and RAL; important immune recovery (CD4 192 cells/mm3) and significant decay of VL to 1.5 log10 were noticed after 1 month. The clinical evolution was unfavorable with the appearance of generalized seizures, movement disturbances, auditory hallucinations and right hemiparesis. CSF exam revealed 2 WBC/mmc, negative bacterial and fungal cultures, HIV-RNA 82 copies/mL (1.90 log10) and positive PCR-DNA JC. Brain MRI showed hyperintense lesions on T2 and FLAIR, hypointense on T1 sequences, localized in fronto-temporal areas, right cerebellum and pons. In evolution there was a continuing worsening of psychiatric and neurological symptoms (with severe tremor, axial hypotonia, repeated seizures, extreme anxiety, sleep disturbances) and important extension of the lesions in subcortical white matter and contrast enhancement that oriented the diagnosis to IRIS-PML. She was treated with high doses of cortisone, antipsychotic drugs and cART was continued, with stable clinical evolution to present. Conclusion: Diagnosis of the neurological complication was difficult due to atypical onset, and the presence of concomitant psychiatric symptoms

MINOCYCLINE INHIBITS PLATELET DEPENDENT NEUROINFLAMMATORY RESPONSE TO HIV INFECTION BY BLOCKING MLK3-P38 MAPK AXIS. Jackson, JW, MS 1, Singh, MV, Ph.D. 1, Singh, VB, Ph.D. 1, Jones, LD, MS 1, Davidson, GA, BS 1, Gorantla, S, Ph.D. 3, Poluektova, LY, Ph.D. 3, Schifitto, G, MD 2, Maggirwar, SB, Ph.D. 1; 1Department of Microbiology and Immunology, University of Rochester Medical Center, Rochester, NY 146422Department of Neurology, University of Rochester Medical Center, Rochester, NY 146423Department of Pharmacology and Experimental Neuroscience, University of Nebraska Medical Center, Omaha, NE 68198.

Platelets are known for their role in hemostasis, wound healing, and inflammation. It is well known that platelets circulate in a more activated state during HIV infection, ultimately contributing to the pathogenesis of secondary disorders such as neurocognitive impairment. Here, using humanized mice, we have established that leukocytes infiltrating into the central nervous system (CNS) correlates with activated platelets in blood, and that platelet depletion reduces HIV-encoded Tat-mediated monocyte migration into the CNS. Thus, the use of adjunctive antiplatelet therapy is of particular importance to combat platelet dependent neuroinflammation during HIV infection. We have shown here that an antibiotic, minocycline, has novel antiplatelet activity, as it reduces levels of platelet-derived soluble CD40L and platelet factor 4 in the plasma of HIV-infected patients. Minocycline reduced activation of platelets, in vitro, following stimulation with thrombin, as measured by ELISA and flow cytometry. Platelet degranulation was also reduced upon exposure to minocycline as shown by mepacrine retention. However, minocycline had no effect on platelet spreading, aggregation, and GPIIb/IIIa activation. Lastly, immunoblot analysis suggests that the antiplatelet activity of minocycline is likely mediated by inhibition of MLK3-p38 MAPK signaling axis. Collectively, this work will illustrate minocycline as a potential therapy to combat platelet dependent neuroinflammation that is often found in HIV infected individuals.

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ENHANCING CIRCUIT SPECIFIC NEUROPLASTICITY IN DEGENERATIVE BRAIN DISORDERS THROUGH EXERCISE. Jakowec, MW, Ph.D. 1, Petzinger, GM, MD 1, Holschneider, D, MD 2, Wang , Z, Ph.D. 2, Halliday, M, BS 1, Stefanko, D, BS 1, Toy, D, BS 1; 1Neurology, University of Southern California, Los Angeles, CA 900332Psychiatry, University of Southern California, Los Angeles, CA 90033.

Exercise and physical activity are critical for maintaining general health for both the body and central nervous system. Our group and others have also shown that exercise can provide neurorestorative benefits in animal models of neurodegenerative brain disorders including Parkinson's disease and Huntington's disease. However, major gaps in our knowledge must be addressed including (i) what forms of exercise are optimal for treating specific brain disorders, (ii) what are the mechanisms underlying physical activity that link peripheral systems with specific brain circuits, (iii) what are the metabolic signals in brain circuits that direct neuroplasticity, and finally (iii) can we translate our findings to treat patients with these neurodegenerative disorders. Ongoing studies in our group are exploring the underlying mechanisms by which exercise can alter disease progression in models of Parkinson's disease and Huntington's disease. Our findings indicate that different forms of exercise can orchestrate circuit and region specific changes in cerebral blood flow, as well as proteins involved in metabolism, and synaptic structure and function. These targeted changes impact both motor and cognitive behaviors reversing deficits seen in our animal models. Our ultimate goal is to translate these findings into improved therapeutic treatment for patients with brain disorders. Our clinical studies in patients with PD are beginning to pursue this goal and provide evidence-based medicine for extending specific modes of physical activity as an integral part of the standard of care.

Supported by NIH, NPF, Gonzalez Fdn.

A NOVEL ROLE OF THE METABOLIC REDOX ENZYME PROLINE OXIDASE IN METHAMPHETAMINE-INDUCED NEUROTOXICITY. Jones, B 1, Dash, S, MS 1, Balasubramaniam, M, Ph.D. 1, Villalta, F, Ph.D. 1, Dash, C, Ph.D. 1, Pandhare, J, Ph.D. 1; 1Center for AIDS Health Disparities Research, Meharry Medical College, Nashville, TN 37208.

Methamphetamine (MA) is a highly addictive psychostimulant drug. MA induced neurotoxicity causes long-lasting effects especially on the dopaminergic neurons in the CNS. Therefore, MA abuse has been linked to increased risk of developing several neurodegenerative diseases, such as Parkinson's disease, HIV-Associated Neurocognitive Disorder (HAND) and others. Despite the well-documented effects of MA on neurotoxicity, the molecular mechanisms remain poorly understood. Several lines of evidence suggest that MA-induced neuronal damage elicits reactive oxygen species (ROS) production and downstream oxidative stress mechanisms, such as apoptosis and autophagy. In this study, we demonstrate a functional role of the metabolic enzyme Proline Oxidase (POX) in MA-induced neurotoxicity. POX, a mitochondrial inner membrane enzyme, catalyzes the first step of proline degradation. Metabolism of proline by POX generates ROS a known inducer of apoptosis and autophagy. Therefore, we hypothesized that POX-dependent ROS may contribute to MA-induced neurotoxicity. To test this hypothesis, we used differentiated SH-SY5Y neuroblastoma cell line as a model to mimic neuronal phenotype. Treatment of SH-SY5Y cells with MA significantly induced POX expression and catalytic activity in a dose dependent manner. Concurrently, MA treatment also resulted in an increase

in ROS levels. Therefore, we are currently investigating the effects of MA-induced increased ROS on neuronal apoptosis as well as autophagy. Taken together, this is the first time a role of POX has been implicated in MA-induced neurotoxicity

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ERADICATING HIV-1 FROM BRAIN COMPARTMENT USING TAT INDUCIBLE CRISPR/CAS9 GENE EDITING STRATEGY. Kaminski, R, Ph.D. 1, Chen, Y, Ph.D. 1, Salkind, J, BS 1, Bella, R, MS 2, Hu, W, MD, Ph.D. 1, Khalili, K, Ph.D. 1; 1Department of Neuroscience and Center for Neurovirology, Temple University School of Medicine, Philadelphia, PA 191402Department of Biomedical, Surgical and Dental Sciences, University of Milan, Milan, 2100.

The presence of long-lived viral latency state is the major obstacle to curative therapy of AIDS. The best characterized latent HIV-1 reservoir resides in resting, memory CD4+ T-cells in peripheral blood and lymphoid tissue. There are several additional viral reservoirs/compartments, with the most prominent: the GALT (gut associated lymphoid tissue) and the brain. Several studies identified macrophages, microglia and astrocytes as a cells hosting dormant HIV-1 in the brain. Using CRISPR/Cas9 gene editing technology to target two unique sequences in U3 region of HIV-1 LTR (called target A and B), we were able to completely eradicate proviral reporter sequences from the genomes of U1 and CHME5/HIV reporter cell lines of monocyte and microglial origin. In the next step we tested and validated our system in primary brain cells: microglia, astrocytes and macrophages. To improve specificity and safety of our therapy we placed Cas9 gene under control of minimal Tat-responding HIV-1 promoter restricting Cas9 expression to HIV-1 infected, activated cells. Spontaneous or drug induced expression of the HIV-1 in the infected brain cells led to stimulation of the TAR/Tat dependent Cas9 expression followed by targeting and cutting of the modulatory U3 region of HIV-1 LTRs (not present in the minimal Tat dependent promoter). Our results suggest that CRISPR/Cas9 system can be used to specifically remove integrated viral sequences from the genomes of HIV-1 infected brain cells providing proof of concept for the future therapy aiming at complete eradication of HIV from the brain compartment.

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INCREASED SOCIAL INTERACTIONS IN RATS FED WITH A HIGH FAT KETOGENIC DIET. Kasprowska, D., Ph.D. 1, Liśkiewicz, A.D., Ph.D. 2, Sługocka , A., MS 3, Barski, J.J., Ph.D. 3; 1Laboratory of Molecular Biology, Faculty of Physiotherapy, The Jerzy Kukuczka Academy of Physical Education, Katowice, 40-0652Department of Physiology, School of Medicine in Katowice, Medical University of Silesia, Katowice, 40-7523Center of Experimental Medicine, Medical University of Silesia, Katowice, PA 40-752.

High fat ketogenic diet (HFKD) is used as a therapy of pharmacologically resistant epilepsy. Seizures are a comorbid condition associated with some neurological disorders e.g. Autism Spectrum Disorders (ASD). However, little is known about the influence of HFKD on various conditions occurring in patients treated with HFKD. Most animal studies use adult subjects of autism rodent models. Considering the significance of developmental period in pathogenesis of disturbed social interactions in ASD it is important to provide comprehensive studies using young animals. However, the ketogenic rodent chow

has many side effects, including total growth inhibition. We have developed a ketogenic rodent chow allowing for its implication in young animals. By simple modification we overcame detrimental effects of this diet which allows us for examination of its prosocial actions. Four weeks old male Long Evans rats were fed with modified HFKD for the next 4 weeks. We observed that rats fed with HFKD showed increased social activity measured as cumulative time of social behaviors, time of partner sniffing and grooming. In order to check if increased activity is limited to social interactions or it results from overall hyperactivity we performed open field test. The results didn't show any difference in mobility of animals fed with modified HFKD, standard HFKD or standard diet. In conclusion, our modification of HFKD allowed us to show its prosocial effect. It may also be used to investigate its effect on physiology, biochemistry and immunology of young subjects in various animal models.

CNS DELIVERY OF MAGNETO-ELECTRO NANOPARTICLES IN NON-HUMAN PRIMATES . kaushik, A, Ph.D. 1; 1Center of Personalized Nanomedicine, Institute of Neuroimmune Pharmacology, Department of Immunology, Herbert Wertheim College of Medicine, Florida International University , Miami, FL 33199.

Our laboratory is exploring least component based targeted drug delivery of nanoformulations across blood-brain-barrier (BBB) to investigate site-specific on-demand therapeutics for the treatment of neurodegenerative diseases. We explored magneto-electric nanoparticles (MENPs, ~20 nm) as a novel potential nanocarries to deliver anti-HIV drugs across in-vitro BBB model for neuroAIDS cure. To translate these novel finding to in-vivo levels, we have demonstrated magnetic guided delivery of MENPs using C57Bl/J mice and studied their BBB transmigration efficacy, bio-distribution and toxicity at optimized dose of 10 mg/kg. The results of our studies showed that MENCs are uniformly distributed across brain, nontoxic and there was no accumulation of inflammatory agents at the target organ i.e. brain or other major organs like kidney, liver, spleen and also there was no neurobehavioral dysfunctioning observed. Based on these preclinical results, we have moved one step ahead and went on testing the CNS delivery efficacy of MENCs at non-human primate level. A dose of 22mg/13kg MENCs suspended in 100ml PBS with a flow rate of 220 mL/hr were injected in baboon via Saphenous vein under static MRI magnetic treatment for 3 hrs. The results of MRI-histopathological studies showed MENCs transmigrate across BBB without showing any neurobehavioral or pathological side-effects. In conclusion, all in vitro & in vivo results showed that MENCs can serve as a potential drug-nanocarrier to deliver therapeutic agents that do not cross brain and will be used to treat CNS diseases.

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BEHAVIOURAL CHANGES IN A MOUSE MODEL OF SCHIZOPHRENIA: THE ROLE OF THE BLOOD-BRAIN BARRIER IN THE DORSAL HIPPOCAMPUS AND MEDIAL PREFRONTAL CORTEX. Kealy, J, Ph.D. 1, Greene, C, BS 1, Campbell, M, Ph.D. 1; 1Smurfit Institute of Genetics, Trinity College Dublin, Dublin, D 2.

Schizophrenia is a neurodevelopmental disorder associated with a variety of cognitive and behavioural symptoms. A number of genetic factors have identified susceptibility loci on chromosome 22 (22q11.21). Located within this region is the gene claudin-5, a fundamental component of the tight junction between endothelial cells in the blood-brain barrier (BBB). Reduced levels of claudin-5 lead to increased permeability of the BBB, which may be associated with some of the symptoms associated with

psychosis. Here, we investigate the behavioural effects of claudin-5 suppression in mice. An adeno-associated virus (AAV-2/9) vector containing a doxycycline-inducible gene encoding shRNA targeting claudin-5 was stereotaxically injected either into the dorsal hippocampus (dHipp) or the medial prefrontal cortex (mPFC). Mice were allowed to recover for 7 days before 2 weeks of doxycycline treatment (2 mg/ml drinking water). Animals subsequently underwent a battery of behavioural tasks covering various aspects of normal behaviour: learning and memory (object recognition task; spatial navigation); anxiety (open field task); depression (forced swim task; splash test); social behaviour (social interaction task); motor co-ordination (neurological severity score; Rota-rod); spatial gating (paired-pulse inhibition) and observation of home cage behaviours. By comparing claudin-5 suppression in dHipp and mPFC across these tasks, it is possible to elucidate the effects of selectively modulating BBB permeability on normal behaviour in mice and in the development of a BBB-based model of psychosis.

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PATTERN RECOGNITION RECEPTORS AND INFLAMMASOME ACTIVATION IN THE BLOOD-BRAIN BARRIER. Krizbai, I.A., MD, Ph.D. 1, Wilhelm, I, MD, Ph.D. 1, Molnár, J, MS 1, Fazakas, C, Ph.D. 1, Haskó, J, MS 1, Kozma, M, BS 1, Nagyőszi, P, Ph.D. 1, Nyúl-Tóth, A, MS 1; 1Biological Research Centre, Institute of Biophysics, Szeged, 6726.

By forming the blood-brain barrier (BBB) cerebral endothelial cells (CECs) are at the interface of the immune and the central nervous systems and thus may play an important role in the functional integration of the two systems. Here we investigated how CECs recognize and respond to pathogen- and damage-associated molecular patterns in order to regulate the functions of the neurovascular unit. In our experiments we used an in vitro BBB model based on the culture of the hCMEC/D3 human cerebral microvascular endothelial cell line. First we detected the expression of several NLRs – including NOD1, NOD2, NLRC4, NLRC5, NLRP1, NLRP3, NLRP5, NLRP9, NLRP10, NLRP12, NLRA and NLRX – in brain endothelial cells. Inflammatory cytokines, such as IFN-γ, TNF-α, and IL-1β had stimulatory effect on the transcription of many of these receptors. Expression of key inflammasome components (NOD2, NLRP3 and caspase-1) along with inflammasome-activated IL-1β could be induced by priming with lipopolysaccharide (LPS) and activation with muramyl dipeptide (MDP). In addition, combined treatment with LPS and MDP resulted in IL-1β secretion in a caspase- and ERK1/2 kinase-dependent manner. Our findings demonstrate that NLRs and inflammasomes can be activated in cerebral endothelial cells, which may confer a yet unexplored role to the BBB in neuroimmune and neuroinflammatory processes.

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STIMULATORY EFFECT OF DESIPRAMINE ON LUNG METASTASIS OF ADENOCARCINOMA MADB 106 CELLS IN STRESS HIGH-SENSITIVE AND STRESS NON-REACTIVE RATS. Marta Kubera1, Beata Grygier1, Danuta Wrona2, Adam Roman3, Piotr Gruca4, Mariusz Papp4, Monika Leśkiewicz1, Bogusława Budziszewska1, Agnieszka Basta-Kaim1, Magdalena Regulska1, Barbara Korzeniak1, Katarzyna Curzytek1, Michael Maes5, Władysław Lasoń1 1 Department of Experimental Neuroendocrinology, 3Departrment of Brain Biochemistry, 4 Laboratory of Behavioral Pharmacology Institute of Pharmacology, Polish Academy of Sciences, 12 Smętna Street, PL 31-343 Kraków, Poland. 2Department

of Animal and Human Physiology, University of Gdansk, Wita Stwosza 59 Str. 80-308 Gdansk, Poland. 5Department of Psychiatry, Faculty of Medicine, Chulalongkorn University, 10330 Bangkok, Thailand

Several scientific clinical trials and experimental studies confirmed the role of psychosocial factors, such as depression, and personality traits in development and/or progress of cancer process but the effect of antidepressant drugs on tumor progress is very poorly recognized. The aim of the present study was to examine the effect of individual reactivity to stress and 24-day desipramine (DES) administration on metastatic colonization of adenocarcinoma MADB 106 cells in the lungs of Wistar rats. Rats were subjected to chronic mild stress (CMS) model of depression for two weeks, and stress high-sensitive (SHS) and stress non-reactive (SNR) rats were selected. Subsequently, all selected animals received i.v. tumor cells and daily i.p. injection of DES (10 mg/kg) or saline. DES administration started immediately after the injection of MADB 106 cells. Chronic DES treatment significantly increased the number of lung metastases in both SHS and SNR rates in comparison to saline-treated appropriate control rats. Following i.v. injection, the MADB 106 tumor cells metastasize only to the lungs. The immune-dependent lung clearance is limited to the first 24 h after tumor cell injection, so immune parameters were studied also 24h after a single DES injection. The increase in lung metastases was connected with DES-induced decrease in the number of TCD8+ and B cells in SHS and SNR rats, NK cell activity in SNR rats, changes in macrophage functional phenotype but not the NK cell number in SHS rats. In the present study, we demonstrated that susceptibility to stress plays a role in the modulatory effect of desipramine on tumor growth.

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EFFECT OF ETHANOL ON THE METABOLISMS OF DARUNAVIR AND ELVITEGRAVIR IN HEPATIC AND MONOCYTIC CELLS: POTENTIAL ROLE OF CYTOCHROME P450 3A4. Kumar, S, Ph.D. 1, Narasimha , M, MS 1, Rahman, MA, MS 1; 1Pharmaceutical Sciences, University of Tennessee Health Science Center, Memphis, TN 38163.

HIV protease inhibitor (PI) darunavir (DRV) and its pharmacoenhancer ritonavir (RTV), and a novel integrase inhibitor elvitegravir (EVG) and its pharmacoenhancer cobicistat (COBI) are part of the first-line antiretroviral therapy (ART) regimens. EVG and DRV are mainly metabolized through cytochrome P450 (CYP) 3A4 enzyme. Previously, we have shown that ethanol alters ART-CYP3A4 interactions with PIs, which are likely to alter their metabolisms. In this study, we characterized the metabolisms of both DRV (+/-RTV) and EVG (+/-COBI) in the presence of 20 mM ethanol in human microsomes and monocytic cells followed by their inhibitory characteristics with CYP3A4. Time kinetics data showed that while ethanol does not alter the apparent half-life (t1/2) of DRV metabolism, it decreases the t1/2 of EVG metabolism. Substrate kinetic results revealed that ethanol decreases the catalytic efficiency for both DRV and EVG metabolisms in the absence and presence of their respective pharmacoenhancers. Inhibition studies demonstrated that ethanol causes an increase in IC50 of EVG, while a decrease in IC50 of EVG. Finally, we showed that the level of both DRV and EVG are altered in the presence of ethanol as we as HIV in monocytic cells, which is consistent with the altered levels of CYP3A4. Taken together, these results suggest that ethanol alters the level of DRV and EVG through CYP3A4 pathway. This finding has clinical significance because alcohol use is highly prevalent in HIV population, and there is no separate guideline for these patients while they are on ART medication.

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INVOLVEMENT OF TNFA IN PROAPOPTOTIC ACTION OF CORTICOSTERONE IN HIPPOCAMPAL ORGANOTYPIC CULTURES. Kurek, A 1, Kucharczyk, M 1, Detka, J 1, Głombik, K, Ph.D. 1, Trojan, E 1, Ludwikowska, A, Ph.D. 1, Cużytek, K 1, Ślusarczyk, J 1, Kubera, M, MD, Ph.D. 1, Budziszewska, B, MD, Ph.D. 1; 1Department of Experimental Neuroendocrinology, Institute of Pharmacology, Polish Academy of Sciences, Kraków, 31-343.

Elevated levels of glucocorticoids (GCs) exert neurotoxic effects and the hippocampus is particularly sensitive to their action. Moreover, increased GCs concentration during the perinatal period permanently modify brain tissue sensitivity to adverse substances acting in adulthood. The aim of this study was to compare cytotoxic effect of corticosterone in the hippocampal organotypic cultures obtained from control and prenatally stressed rat and to assess TNF-α contribution in this hormone action. The hippocampal organotypic cultures were prepared from brains of 7-day old offspring of control stressed mothers. Corticosterone and/or glutamate were added to culture medium for 24h or 72h and next necrotic and apoptotic markers as well as concentrations of growth factors and TNF-α were determined. It has been found that corticosterone didn't induce necrosis, but increased apoptotic markers. In the 24h culture corticosterone and glutamate increased growth factors and TNFα expression, whereas after 72h only expression of the TNF-α remained high. Moreover, corticosterone action on caspase-3 activity and TNF-α expression was stronger in the hippocampus from prenatally stressed than control rats. These data indicate that prenatal stress increased hippocampal sensitivity to the pro-apoptotic action of corticosterone and proinflammatory cytokine TNF-α appears to be involved in this effect.

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INVOLVEMENT OF TNFA IN PROAPOPTOTIC ACTION OF CORTICOSTERONE IN HIPPOCAMPAL ORGANOTYPIC CULTURES. Kurek, A 1, Kucharczyk, M 1, Detka, J 1, Głombik, K, Ph.D. 1, Trojan, E 1, Ludwikowska, A, Ph.D. 1, Cużytek, K 1, Ślusarczyk, J 1, Kubera, M, MD, Ph.D. 1, Budziszewska, B, MD, Ph.D. 1; 1Department of Experimental Neuroendocrinology, Institute of Pharmacology, Polish Academy of Sciences, Kraków, 31-343.

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IMBALANCE OF CD4+ T HELPER AND T REGULATORY LYMPHOCYTE SUBSETS IN PERIPHERAL BLOOD OF PARKINSON'S DISEASE PATIENTS. Kustrimovic, N, Ph.D. 1, Aleksic, I, BS 1, Rasini, E, BS 1, Legnaro, M, BS 1, Blandini, F, MD 2, Comi, C, MD 3, Bono, G, MD 4, Pacchetti, C, MD 2, Marino, F, Ph.D. 1, Cosentino, M, MD, Ph.D. 1; 1Center of Research in Medical Pharmacology, University of Insubria, Varese, 211002Research Center for Parkinson's Disease, Neurological Institute "C. Mondino", Pavia, 271003Movement Disorders Center, Divisione di Neurologia, Ospedale Maggiore , University of Piemonte Orientale, Novara, 281004Centre for Parkinson's Disease and Movement Disorders , Ospedale di Circolo of Varese , Varese, 21100.

Increasing evidence supports the involvement of the peripheral adaptive immune system in the pathogenesis of Parkinson's disease (PD), the second most common neurodegenerative disease affecting 7-10 millions people worldwide. To investigate CD4+ T cell subsets in PD, we enrolled PD patients (n = 61, age (mean±SD): 69.6±8.9 years) and healthy subjects (HS, n = 42, age 68.0±10.1 years). By means of flow cytometry, we assessed the frequency of T helper (h)1/2/17, and of naïve (n) and activated (a) T regulatory cells (Treg). PD patients had less total lymphocytes (1767.7±507.1 10e6cells/L in PD patients vs. 2059.1±586.3 10e6cells/L in HS, P = 0.008), mainly due to reduced CD4+ T cells (785.6±263.4 vs. 975.5±373.0, P = 0.003). PD patients had less Th2 (49.0±23.4 vs. 72.5±38.6, P = 0.001), Th17 (57.2±27.6 vs. 90.0±47.8, P<0.001), Th1/Th17 (87.5±48.2 vs. 114.8±70.0, P = 0.045), aTreg (22.2±6.9 vs. 34.2±14.5, P<0.001), and nTreg cells (15.8±5.8 vs. 21.5±9.8, P = 0.004) in comparison to HS. In percentage of total CD4+ T cells, PD patients had less Th2 (6.3±2.5% vs. 7.7±3.7%, P = 0.05), Th17 (7.4±2.8% vs. 9.6±4.2%, P = 0.006) but more Th1 (18.9±8.3% vs. 14.3±6.0%, P = 0.009). PD patients thus have complex changes in peripheral immunity, possibly suggesting the prevalence of a Th1 proinflammatory profile. Therapeutic strategies aimed at reducing neurotoxic Th1 and increasing Treg/Th2 neuroprotective immunity could be beneficial in PD.

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HIV-1 VPR-TRANSFECTED ASTROCYTES CAUSE MORPHOLOGICAL CHANGES IN NEURONS. Lamboy, R 1, Noel, R 1; 1Ponce Research Institute, Ponce Health Sciences University, Ponce, PR 00716.

The human immunodeficiency virus (HIV-1) infects and destroys the cells of the immune system, affecting peripheral organs and the brain. HIV-infected individuals can suffer from a wide range of neurocognitive impairments collectively termed HIV-associated neurocognitive disorders (HAND). HIV induces neurotoxicity through the expression of several individual viral proteins including viral protein R; a neurotoxin that can affect the glial cells and neurons. The aim of this study is to determine the capacity of Vpr-transfected astrocytes to cause neurotoxicity and affect the morphology of neurons in co-culture. To test Vpr neurotoxicity, primary rat astrocytes were transfected with a plasmid-encoding

Vpr-GFP and were grown at different time points in co-culture with rat cortical neurons. After 48 and 72 hours, no significant morphological changes, as assessed by filamentous actin staining, were observed in neurons co-cultured with Vpr-transfected astrocytes. These data suggest that Vpr does not cause large-scale morphological changes to neurons in co-culture. The fact that such damage was observed in vivo suggests that astrocytes producing Vpr may need to be in direct contact with the neurons or may require the presence of other cells such as microglia. It is possible that the damage is more subtle and future experiments will probe the impact of Vpr on different synaptic proteins to improve our understanding of its neurotoxic capacity.

Supported by MBRS-RISE program (R25GM082406), Dr. Noel Lab (GM106970), and the PHSU-PRI MAGIC Lab (MD007579).

THE ROLE OF AUTOPHAGY IN HIV-1 TAT INDUCED NEURODEGENERATION USING BECLIN-1 HETEROZYGOUS MOUSE BEHAVIOR MODEL. Lapierre, J, MS 1, Vinerean, H 1, Rodriquez, M, Ph.D. 1, Nair, M, Ph.D. 1, El-Hage, N, Ph.D. 1; 1Department of Immunology, Florida International University College of Medicine, Miami, FL 33199.

Evidence suggests that HIV-1 induces neurological impairments leading to the development of HIV-associated neurocognitive disorders (HAND). Moreover, intravenous opiate drug users account for 30% of AIDS cases in the USA who frequently show acceleration to AIDS dementia. Inhibition of neuronal autophagy has previously been linked to neurodegeneration seen in the HIV-1-infected brain with representative neuronal loss. Specifically, the striatum brain region which is responsible for motor coordination is disrupted by HIV and opioids. In this study, Becn1-deficient mice which are heterozygous for the Becn1 allele and show reduced autophagy were exposed to both HIV-Tat and morphine to assess alterations in behaviors and correlated immunohistochemistry associated with neuroAIDS brain pathology. Intrastriatal injections of HIV-Tat protein followed by insertion of subscapular time-release morphine pellets were performed in vivo using the Becn1-deficient mice and C57BL/6J wildtype controls. Motor function, strength, and coordination was gauged using tests such as grip strength, horizontal bars, and rotarod. No differences were seen in the untreated wildtype C57BL/6J and Becn1-deficient mice; however, significant differences were noted between the groups with intrastriatal exposure to Tat. No significant behavioral differences between the strains were noted when treated with combined Tat and morphine. This suggests that autophagy is cytoprotective and lack of this pathway exacerbates Tat-induced pathology, while the interactive effects of morphine may not converge at this pathway.

Supported by NIH.

DIMETHYL FUMARATE EFFECTS ON CORTICOSTERONE RESPONSE IN YOUNG RATS WITH ALZHEIMER'S DISEASE MODEL. Laska, Ewa 1, Grzybowska, Maria 1, Podlacha, Magdalena 1, Chwiej, Monika 1, Majkutewicz, Irena 1, Wrona, Danuta 1; 1Department of Animal and Human Physiology, University of Gdansk, Gdansk, 80-309.

An effect of dimethyl fumarate (DMF), antioxidant and immunosuppressive drug, on plasma corticosterone (CORT) response in young (4 mounth-old) Wistar rats with streptozotocin (STZ)-induced

sporadic form of Alzheimer's Disease (sAD) model, differing in locomotor response to novelty/stress susceptibility (high (HR) or low (LR) responders). HRs and LRs were divided into groups: STZ DMF (intracerebroventricular (i.c.v.) injection of STZ, 0.4% DMF fodder), VEH DMF (i.c.v. injection of vehicle, 0.4% DMF fodder), STZ CTR (i.c.v. injection of STZ, standard fodder), VEH CTR (i.c.v. injection of vehicle, standard fodder). Blood samples were taken for determination of plasma CORT concentration (RIA). A significantly ($P<0.05$) higher level of CORT in the non-divided behaviorally (156.22±70.75 ng/ml) and the HRs (187.90±72.21 ng/ml) or LRs (118.20±51.81 ng/ml) in the STZDMF group rather than control VEHDMF group (non-divided: 84.52±41.72 ng/ml and HRs: 106.79±46.94 ng/ml or LRs: 54.85±22.45 ng/ml) was observed. Moreover, DMF significantly ($P<0.05$) decreased plasma CORT concentration in the control VEHDMF group (84.52±41.72 ng/ml) and the LRs but not HRs (LRs: 54.85 ng/ml) as compared to the controls (VEHCTR:135.12±59.20 ng/ml; LRs: 106.98±26.01 ng/ml) with a significant ($P<0.05$) HRs vs. LRs difference. It suggests that such an immunosuppressive drug as DMF does not influence corticosterone response in young rats with sAD model. However, it decreases corticosterone level in the controls, in particular, in the rats with a lower behavioral activity/stress susceptibility(LRs).

METHAMPHETAMINE-MEDIATED OXIDATIVE STRESS CONTRIBUTES TO THE DYSREGULATION OF AUTOPHAGIC FLUX OF ASTROCYTES. Li, J-L, Ph.D. 1, Onyeachu, V, BS 1, Wang, X, Ph.D. 1, Ma, T-C, MS 3, Ho, W-Z, MD, M.Ph. 2; 1Department of Pathology and Laboratory Medicine, Temple University Lewis Katz School of Medicine, Philadelphia, PA 191402Center for Substance Abuse Research, Temple University Lewis Katz School of Medicine, Philadelphia, PA 191403Animal Biosafety Level III Laboratory at the Center for Animal Experiment, State Key Laboratory of Virology, Wuhan University School of Basic Medical Sciences, Wuhan, CA 430071.

Methamphetamine (METH) has been implicated as a comorbidity risk for neurocognitive dysfunction in HIV-positive individuals. In the present study, we investigated the effect of METH on autophagy of astrocytes, a crucial cellular process in maintaining the cell homeostasis. The expression of endogenous autophagy-related genes (ATGs) was examined by western blotting and quantitative real-time PCR. The effect of METH on the microtubule light-chain B (MAP1LC3B)-puncta number was measured by fluorescence microscopy. Cellular oxidative stress was quantified by the CellROX probe. METH treatment of human astrocytes increased the mRNA expression of Beclin1 and LC3, two key ATGs for the formation of autophagosome. METH triggered the endogenous expression and conversion of MAP1LC3B-I to MAP1LC3B-II and increased the LC3 puncta in pEGFP-LC3 transfected astrocytes. METH-induced autophagy could be blocked by the phosphatidylinositol-3 kinase (PI3K) inhibitor 3-MA. On the contrary, in the presence of Bafilomycin A1, a proton pump inhibitor that blocks the autophagosome (AP)-lysosome fusion, METH did not alter the levels of LC3-II, indicating that the upregulated conversion was due to the impaired autophagic flux. Investigation of the underlying mechanism for METH-induced autophagy showed that METH treatment increased the cellular levels of oxidative stress, the inhibition of which could attenuate MAP1LC3B conversion. Collectively, these findings suggest that METH-mediated oxidative stress contributes to the dysregulation of autophagic flux of astrocytes.

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INVOLVEMENT OF VOLTAGE-DEPENDENT K+ CHANNEL 1.3 IN HIV TAT-INDUCED OLIGODENDROCYTE/MYELIN INJURY. Liu, H 1, Xiong, H, MD, Ph.D. 1; 1Pharmacology and Experimental Neuroscience, University of Nebraska Medical Center, Omaha, NE 68198-5880.

Brain white matter (WM) is composed of neuronal axons ensheathed by oligodendrocyte(Ol)s, the myelin-forming cells. HIV-1 brain infection caused myelin/WM injury is commonly seen in patients with all forms of HIV-1-associated neurocognitive disorders. Ols are sensitive to HIV-1 viral trans-activator of transcription (Tat) and myelin damage is associated with Ol number decrease. Activation of voltage-gated K+ (KV) channels produces apoptosis in various types of cells, including HIV-1 protein-treated neurons and microglia. KV1.3 is the most predominant KV channel in Ols and potentially involved in regulation of Ol development. We hypothesize that Tat induces Ol injury through KV1.3 activation. To test the hypothesis, we studied the involvement of Ol KV1.3 in Tat-induced Ol/myelin injury in vitro and ex vivo. Application of Tat to primary rat Ol cultures increased KV1.3 current as revealed by whole-cell patch-clamp recording, leading to myelin basic protein (MBP) reduction and Ol apoptosis, which were prevented by specific KV1.3 blockers 5-(4-phenoxybutoxy) psoralen and Margatoxin, or KV1.3-siRNA knockdown. Tat-caused myelin injury was validated in cultured rat brain slices, particularly in corpus callosum and striatum, which was also blocked by aforementioned KV1.3 antagonists. Tat interacts with KV1.3 was revealed by co-immunoprecipitation of Tat with KV1.3 over-expressed HEK293. Taken together, our results suggested that Tat induces WM damage by KV1.3-mediated Ol/myelin injury. Thus, blockade of Ol KV1.3 is a potential therapeutic strategy for HIV-1-associated WM damage.

INVOLVEMENT OF VOLTAGE-GATED K CHANNELS IN METHAMPHETAMINE POTENTIATION OF HIV-INDUCED MICROGLIA NEUROTOXICITY. Liu, J, MD, Ph.D. 1, Liu, H, MS 1, Xu, E, MS 1, Xiong, H, MD, Ph.D. 1; 1Department of Pharmacology and Experimental Neuroscience, University of Nebraska Medical Center, Omaha, NE 68198.

HIV brain infection causes microglia (MG) activation and release of proinflammatory molecules leading to HIV-associated neurocognitive disorders (HAND). Methamphetamine (Meth) abuse exacerbates HAND. The mechanisms underlying such an exacerbation are not fully understood. Voltage-dependent potassium (Kv) channels have recently gained attention in the regulation of MG functionality and MG express several types of Kv channels such as outward delayed rectifiers Kv1.5 and Kv1.3. We hypothesize that Meth potentiates HIV-induced MG neurotoxicity via activation of MG Kv1.3. To test this hypotheses, we studied co-morbid effects of Meth and HIVgp120 (gp120) on MG Kv1.3 expression, KV1.3 current and involvement of Kv1.3 in MG production of neurotoxins. Our results revealed that Meth potentiated gp120 enhancement of KV1.3 protein expression, KV1.3 current and MG production of neurotoxins leading to neuronal apoptosis, which were blocked by pretreatment of MG with specific KV 1.3 channel blocker 5-(4-Phenoxybutoxy)psoralen (PAP), or by broad spectrum KV channel blocker, 4-AP, indicating an involvement of KV1.3 in Meth/gp120-induced MG neurotoxic activity. Our results demonstrated an involvement of MG Kv1.3 in the mediation of a comorbid effect of Meth/gp120 on MG neurotoxic activity.

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EGCG PREVENTS MUCOSAL SIV/SHIV TRANSMISSION OF MACAQUES. Liu, Jinbiao, MS 1, Li, Jieliang, Ph.D. 2, Zhuang, Ke, Ph.D. 1, Wang, Xu, Ph.D. 2, Xian, Qiaoyang, MS 1, Wang, Yong, BS 1, Liu, Hang, MS 1, Zhou, Runhong, MS 1, Zhou, Li, Ph.D. 1, Ma, Tongcui, MS 1, Sun, Li, MS 1, Li, Xiangdong, MS 1, Guo, Deyin, Ph.D. 3, Wu, Jianguo, Ph.D. 3, Ho, Wenzhe, MD, M.Ph. 2; 1Animal Biosafety Level III Laboratory at the Center for Animal Experiment, Wuhan University, Wuhan, 4300712Department of Pathology and Laboratory Medicine, Temple University School of Medicine, Philadelphia, PA 191403The State Key Laboratory of Virology, Wuhan University, Wuhan, 430071.

As a portal of entry for HIV-1 infection, the sexual mucosal surface is a major site of the viral transmission. Thus, interventions that target at this site are likely to be the most effective in HIV-1 prevention. Epigallocatechin gallate (EGCG), the major active component of green tea, has been shown to have anti-inflammation and anti-HIV-1 properties. Here, we examined the protective effect of EGCG against intra-rectal SIV (mac251) infection of cynomolgus macaques (n=8) and intra-rectal SHIV (SF162P3N) infection of rhesus macaques (n=16). We demonstrated that the intra-rectal administration of EGCG prior to infection could protect (>92%) the animals from a serious of repeated intra-rectal SIV or SHIV challenges with a low-dose (10TCID50). All the protected animals showed no evidence of systemic and mucosal viral infections as demonstrated by the absence of viral RNA, DNA and antibodies. In contrast, all the animals in control group became infected after repeated challenges with SIV (a median of 6, range of 3-8 times for cynomolgus) or SHIV (a median of 2.5, range of 1-8 times for rhesus). The critical mechanisms of the EGCG-mediated prevention of SIV or SHIV mucosal transmission are its competition with gp120 for CD4 receptor binding and the suppression of the mucosal immune activation. These data support further clinical evaluation and development of EGCG as a topical agent for preventing sexual mucosal transmission of HIV-1.

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METABOLIC REMODELING IN GLIOMA T98G CELL CULTURE: A SIMPLE TRIAL TO WEAKEN THE MALIGNANT NATURE OF CANCER CELLS?. Ludyga, T.M., MS 1, Bielecka-Wajdman, A.M., Ph.D. 1, Obuchowicz, E.M., MD, M.Ph. 1; 1Department of Pharmacology, School of Medicine in Katowice, Medical University of Silesia, Katowice, 40-752.

Glioblastoma multiforme is the most aggressive primary tumor in humans. Recently, some progress has been made in the investigation of the targeted inhibition of oncogenic signaling pathways. However, in patients with malignant glioma, the newly designed treatments produce only a transient effect and support development of resistance to standard therapy. Therefore now, a change in the direction of studies concerning glioma biology is necessary. In the newest studies, it has been proposed to look for a possibility to modulate hyperglycemic glioma metabolism that is responsible for keeping its malignant phenotype. In an in vitro study, we induced metabolic remodeling of T98G human glioma cells by an attenuation of the glycolysis intensity and inhibition of the release of Krebs cycle acid metabolites (important markers showing the spread of cancer). Because the main idea of all our studies is to conduct the experiments in the conditions reproducing as much as possible the complex glioma microenvironment, we performed our research in different oxygen conditions. The aim of the study was to investigate for the first time whether and to what extent the changes in glucose concentration in the

medium would influence the level of lactate and pyruvate release from glioma cells, activity of Hif transcription factor and sensitivity to temozolomide in comparison to the effect of glycolysis suppression elicited by 2-deoxy-D-glucose. Our preliminary results showed that access to glucose can determine malignancy and chemosensitivity of glioma cells to temozolomide.

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ACUTE ADMINISTRATION OF ENDOTHELIAL-TARGETED CATALASE ATTENUATES NEUROPATHOLOGY AND CORTICAL MICROGLIA ACTIVATION IN TRAUMATIC BRAIN INJURY (TBI). Lutton, EM 1, Razmpour, R 1, Seasock, M 1, Merkel, SF 1, Andrews, AM, Ph.D. 1, Shuvaev, V, Ph.D. 2, Muzykantov, VR, MD, Ph.D. 2, Ramirez, SH, Ph.D. 1; 1Department of Pathology and Laboratory Medicine, Lewis Katz School of Medicine at Temple University, Philadelphia, PA 191302Institute of Translational Medicine and Therapeutics, University of Pennsylvania, Philadelphia, PA 19104.

TBI is a major clinical and social concern that contributes to one third of all injury related deaths in the US. Secondary mechanisms of injury in TBI, such as inflammation, are points at which intervention may improve functional recovery. Current treatment strategies for TBI are supportive, and the pathophysiology is not fully understood; however, evidence suggests that reactive oxygen species (ROS) and oxidative stress propagate blood-brain-barrier (BBB) hyperpermeability and inflammation following TBI. Such inflammation is associated with activated microglia that have been shown to persist for years after injury in the human brain. The novel use of endothelial-targeted catalase in TBI is hypothesized to quench ROS at their source to limit inflammatory activation and protect BBB function. Preliminary data demonstrates a consistent time dependent increase in vascular expression of endothelial ICAM-1 after TBI. To evaluate targeted antioxidant enzyme efficacy in TBI, catalase was conjugated to anti-ICAM-1 antibodies and administered to 6wk old C57BL6 mice 30min after moderate controlled cortical impact injury. Results suggest that administration of catalase targeted to ICAM-1 reduces neuroinflammatory indices and BBB permeability. Specifically, the study of microglia in situ by multiphoton microscopy following TBI revealed that treatment with anti-ICAM-1/catalase attenuates microglia transition to an activated phenotype. These results demonstrate an effective proof-of-concept approach to acute TBI management that may also be applicable to other neuroinflammatory conditions.

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KUNITZ INHIBITOR (KI) INHIBITS HIV INFECTION OF MACROPHAGES THROUGH JAK/STAT PATHWAY. Ma, T-C, MS 1, Wang, X, MS 1, Li, J-L, Ph.D. 1, Zhuang, K, Ph.D. 2; 1Department of Pathology and Laboratory Medicine, Temple University Lewis Katz School of Medicine, Philadelphia, PA 191402School of Basic Medical Sciences, Wuhan University, Wuhan, 430071.

The Kunitz inhibitor (KI) is a soybean-derived trypsin inhibitor that has anti-inflammatory effect. It is known that macrophages are involved in HIV-mediated inflammation and immune activation, which facilitate HIV disease progression. This study evaluated the effect of KI on HIV infection of peripheral blood monocyte-derived macrophages. We showed that KI dose-dependently inhibits HIV replication in macrophages without cytotoxicity. Investigation of the mechanism(s) of KI action on HIV showed that KI-treated macrophages expressed the multiple IFN stumulated genes (ISGs) that have antiviral activities,

including myxovirus resistance protein 2 (Mx2), Oligoadenylate synthetase (OAS-1), virus inhibitory protein (Viperin), RNA-dependent kinase R (PKR), IFN-stimulated gene 15 (ISG15) and ISG56. In addition, KI also enhanced the expression of APOBEC3G/3F, and Tetherin, the HIV restriction factors. The induction of the ISGs and the HIV restriction factors by KI, however, was not mediated through IFN-alpha and IFN-beta, as KI had little effect on the expression of these antiviral cytokines. In contrast, KI treatment of macrophages significantly induced the production of IL-27, an activator of Jak/STAT signaling pathway. These findings indicate the therapeutic potential of KI in the treatment of HIV disease.

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HUMAN IMMUNODEFICIENCY VIRUS INFECTION CAUSES MITOCHONDRIAL DYSFUNCTION IN ASTROCYTES. Malik, S, Ph.D. 1, Eugenin, EA, Ph.D. 1; 1Public Health Research Institute, New Jersey Medical School-Rutgers University, Newark, NJ 071032Department of Microbiology, Biochemistry and Molecular Genetics, New Jersey Medical School-Rutgers University, Newark, NJ 07103.

HIV-1 is a major public health concern with 34 million people infected with the virus. Although significant viral suppression is achieved with cART, HIV-Associated Neurocognitive Disorders are still observed in 50-60% cases. The major sites for HIV replication in the brain are microglia/macrophages, however a small population of astrocytes also becomes infected. Previously our laboratory has shown that mitochondrial dysfunction post-HIV infection generates apoptotic signals (Ca2+ and Inositol triphosphate (IP3)) that travel from HIV infected astrocytes to surrounding uninfected cells through gap junction channels resulting in apoptosis of uninfected cells. Our previous data supports the hypothesis that mitochondrial involvement is essential for bystander apoptosis. In the present study, we observed that HIV infection induces mitochondrial fusion. Also, mitochondrial oxygen consumption rate as well as the basal metabolic rate was reduced post-HIV infection. Moreover, respiratory capacity of U87 cells infected with HIV was also reduced as compared to uninfected cells. In addition, U87 cells infected with HIV had elevated levels of IP3 as opposed to uninfected cells. Although there was no apoptosis in HIV-infected cells, reduced cellular respiratory capacity indicates mitochondrial dysfunction and possible perturbation of calcium signaling in infected cells. Our data provides novel insights into mitochondrial abnormalities in astrocytes and contributes in delineating their role in NeuroAIDS.

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INTERLEUKIN-1BETA INDUCES LONG-TERM EFFECTS ON THE DEVELOPMENT OF GLUTAMATERGIC NEURONS. Marchetti, N 1, Boraso, M, Ph.D. 1, Galli, CL, Ph.D. 1, Marinovich, M, Ph.D. 1, Viviani, B, Ph.D. 1; 1Department of Pharmacological and Biomolecular Sciences, University of Milan, Milan, 20133.

Epidemiological and experimental studies suggest a link between dysregulated cytokines (IL-1β or TNF-α) production during the perinatal period and the onset of cognitive disorders or the increased vulnerability to neurodegenerative diseases later in life. Due to the ability of IL-1β to modulate function and distribution of the NMDA receptors, we evaluated if a short exposure of developing neurons (3,7,14 days in vitro (DIV)) to IL-1β might result in an altered development of the glutamatergic system. IL-1β specifically reduces GluN2A expression at 21DIV in primary hippocampal neurons exposed to the

cytokine at 3 and 7DIV. This results in an increased GluN2B/GluN2A ratio, favouring the GluN2B-containing NMDARs. This effect is not evident when neurons are exposed at 14DIV, suggesting that IL-1β influences neuronal development only in a specific time window. Moreover, we evaluated the contribution of GluN2A and GluN2B subunits on the calcium increase triggered by NMDA by using specific inhibitors for each subunit. In accordance with protein expression, we observed that in hippocampal neurons pre-treated with IL-1β at 7DIV, 60% of the NMDA-induced calcium increase at 21DIV depends on the GluN2B subunit, while this portion is significantly lower in controls. Our findings suggest that a transient increase of IL-1β during neuronal development leads to "long-term" functional and structural alteration of the glutamatergic system providing a molecular link between neuroinflammation and "long-term" alteration of neuronal activity.

METHAMPHETAMINE ABUSE AFFECTS GENE EXPRESSION IN BRAIN-DERIVED MICROGLIA OF SIV-INFECTED MACAQUES TO ENHANCE INFLAMMATION AND PROMOTE VIRUS TARGETS. Marcondes, MC, Ph.D. 1, Najera, JA, Ph.D. 1, Bustamante, EA, MD 1, Bortell, N, BS 1, Morsey, B, Ph.D. 2, Fox, HS, MD, Ph.D. 2, Ravasi, T, Ph.D. 3, Marcondes, MC, Ph.D. 1; 1Molecular and Cellular Neurosciences Department, The Scripps Research Institute, La Jolla, CA 920372Department of Pharmacology and Experimental Neuroscience, university of Nebraska Mediical Center, Omaha, NE 681983Division of Chemical and Life Sciences and Engineering, King Abdullah University of Science and Technology, Thuwal, 23955.

Abstract Background: Methamphetamine (Meth) abuse is a major health problem linked to the aggravation of HIV- associated complications, especially within the Central Nervous System (CNS). Within the CNS, Meth has the ability to modify the activity/ function of innate immune cells and increase brain viral loads. Here, we examined changes in the gene expression profile of neuron-free microglial cell preparations isolated from the brain of macaques infected with the Simian Immunodeficiency Virus (SIV), a model of neuroAIDS, and exposed to Meth. We aimed to identify molecular patterns triggered by Meth that could explain the detection of higher brain viral loads and the development of a pro-inflammatory CNS environment in the brain of infected drug abusers. Results: We found that Meth alone has a strong effect on the transcription of genes associated with immune pathways, particularly inflammation and chemotaxis. Systems analysis led to a strong correlation between Meth exposure and enhancement of molecules associated with chemokines and chemokine receptors, especially CXCR4 and CCR5, which function as co-receptors for viral entry. Conclusions: Meth enhances the availability of CCR5-expressing target cells for SIV in the brain, in correlation with increased viral load. This result suggests that changes caused by Meth in microglial cells play a significant role in the susceptibility to the infection and to the outcome of the CNS inflammatory pathology associated with SIV in macaques and HIV in humans.

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ENDOGENOUSLY PRODUCED NEF CHANGES NEURONAL MORPHOLOGY IN A CO-CULTURE SYSTEM. Martinez-Orengo, N, BS 1, Vargas, V 2, Noel, R, Ph.D. 1; 1Ponce Research Institute, Ponce Health Sciences University, Ponce, PR 007162Department of Biology, University of Puerto Rico, Ponce, PR 00734 .

Several HIV-1 proteins are pathogenic for cells without the need for active viral replication. This is the case of Nef, an early HIV-1 accessory protein, which causes learning impairment and damage to cells and tissues in our rat model of Nef neuropathology. There is also increased expression of Transforming Growth Factor beta (TGFb). We want to study the effect of astrocyte-produced Nef on neuronal fate and the role of TGFb in Nef-induced neuropathogenesis. To investigate if Nef increases TGFb and alters neuron structure, we measured MAP2, synaptophysin and TGFb expression. Astrocytes were transfected with a vector expressing either Nef or GFP (control group) and co-cultured with neurons for 48 and 72 hours. Astrocyte lysates and supernatants were collected for protein quantification and western blotting; neuronal markers were assessed by immunofluorescence. Neurons exposed to astrocytes expressing Nef presented higher levels of MAP2 when compared to controls, which was more noticeable at 72 hours. TGFb expression was more intense in Nef treated cells compared to control. Synaptophysin expression was decreased in neurons exposed to Nef suggesting synaptodendritic damage. This data indicates that Nef expression in neighboring astrocytes increases MAP2 levels in neurons in a time dependent manner. We think that Nef may cause loss of neuron function without causing neuronal loss via modulation of TGFb pathway. We can conclude that direct contact is not needed in order for Nef to have an effect on neurons so long as the extracellular fluid is in contact with both cell types.

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BLOOD-BRAIN BARRIER COMPROMISE BY HIV-1 TAT AND OPIOIDS IN AN IN VITRO MODEL. Maubert, ME, BS 1, Kercher, KA, MS 1, Strazza, M, Ph.D. 1, Pirrone, V, Ph.D. 1, Lin, W, BS 2, Feng, R, Ph.D. 2, Wigdahl, B, Ph.D. 1, Nonnemacher, M, Ph.D. 1; 1Department of Microbiology and Immunology, Institute for Molecular Medicine and Infectious Disease, Drexel University College of Medicine, Philadelphia, PA 191022Department of Biostatistics and Epidemiology, Center for Clinical Epidemiology and Biostatistics, University of Pennsylvania School of Medicine, Philadelphia, PA 19104.

Illicit drug use is a known comorbidity in exacerbation of disease in human immunodeficiency virus type 1 (HIV-1)-infected individuals. Importantly, opioid abuse within this population confounds disease progression in multiple ways, including increased viral replication and peripheral viral load, and enhanced incidence and severity of neurocognitive impairment including dementia, as compared to non-users. In addition, many of the pathological observations made in HIV-1-associated neurocognitive disorders (HAND) have been attributed to compromise of blood-brain barrier (BBB) integrity, and multiple viral proteins have been implicated in deregulation of the BBB. Previous studies suggest that exposure to both HIV-1 Tat protein and mu-opioids perturbs BBB homeostasis and permeability in primary cells, including an increased pro-inflammatory state, as well as augmented cellular transmigration, and enhanced 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 or morphine exposure on BBB compromise. Changes in mRNA transcripts of tight junction proteins (TJP) were observed throughout the course of exposure. Differences in TJP expression and localization were also observed at the protein level following cellular fractionation and western immunoblot analysis. These studies demonstrate that exposure to Tat or morphine compromises BBB integrity by inducing alterations in molecular expression at both the mRNA and protein levels.

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MINDING THE GAP: PROGRESSION OF TEMPORAL PROCESSING DEFICITS IN THE HIV-1 TRANSGENIC RAT. McLaurin, K.A., BS 1, Booze, R.M., Ph.D. 1, Moran, L.M., Ph.D. 1, Li, H., MD, Ph.D. 1, Mactutus, C.F., Ph.D. 1; 1Program in Behavioral Neuroscience, Department of Psychology, University of South Carolina, Columbia, SC 29208.

Animal models, such as the HIV-1 transgenic (Tg) rat, which expresses 7 of the 9 HIV-1 genes, are ideal for investigating the effect of long-term HIV-1 viral exposure, as seen in pediatric HIV/AIDS, on neurocognitive deficits. Gap detection (gap-PPI), a translational experimental paradigm, was used to study the progression of temporal processing deficits in the HIV-1 Tg rat. The gap-PPI experimental paradigm was conducted using intact male and female Fischer HIV-1 Tg and F344 control rats in a longitudinal experimental design. Gap-PPI was conducted at 30-day intervals from postnatal day (PD) 30 to PD 150. The developmental trajectory of the gap-PPI profile was altered in the HIV-1 Tg rat relative to controls assessed using peak ASR amplitude for startle response (0 and 4000 msec interstimulus intervals (ISI)) and mean area under the amplitude curve measurements. A differential sensitivity to the manipulation of ISI was also observed in the HIV-1 Tg rat. Animals can be correctly classified (91.7%) based on genotype using multiple ISI measures of gap-PPI across development. Temporal processing deficits observed in the HIV-1 Tg rat, as assessed using gap-PPI resemble sensorimotor gating deficits commonly exhibited in HIV-1 seropositive individuals. Understanding the altered progression of temporal processing and insensitivity to the manipulation of ISI provides vital information regarding the effect of long-term exposure to HIV-1 viral proteins on temporal processing deficits in HIV-1 seropositive individuals.

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HIGHER HYPOCRETIN (OREXIN) LEVELS CORRELATES WITH BETTER MOTOR SKILLS IN HIV+ WOMEN. Menéndez-Delmestre, R 1, Rodriguez-Benitez, R 2, González, C 1, Matos, M 1, Noel, RJ 3, Wojna, V 1; 1NeuroAIDS , University of Puerto Rico Medical Sciences Campus, San Juan, PR 009262General Social Sciences, University of Puerto Rico Rio Piedras Campus, San Juan, PR 009263Biochemistry Department, Ponce School of Medicine, Ponce, PR 00716.

HIV-associated neurocognitive disorders (HAND) are characterized by cognitive, behavioral and motor dysfunctions. Brain areas involved in motor control are directly modulated by the hypocretin (orexin) system. In this study we investigated the correlation of hypocretin-1 (hcrt-1) levels with cognitive performance and motor skills in HIV-positive women. In a retrospective study, we measured serum and CSF hcrt-1 levels of 35 HIV-positive women without a history of drug abuse. Cognitive performance was determined using the HAND criteria, where 8 cognitive domains were evaluated, each with two individual tests. CSF and serum hcrt-1 levels were determined using the fluorescent immunoassay kit (Phoenix Pharmaceuticals). Non-Parametric statistics were used to determine correlation ($p<0.05$). A positive correlation was observed between the hcrt-1 ratio (CSF/serum) and the motor skills domain performance of HIV-positive women ($p=0.041$; rho= 0.348). Among individual neuropsychological tests,

the hcrt-1 ratio (CSF/serum) had a positive correlation with the grooved pegboard non-dominant hand test performance (p=0.023; rho= 0.384). No correlation was observed between hcrt-1 levels and other cognitive domains, age, education, body mass index, or viral profile. The positive correlation between the hcrt-1 ratio (CSF/serum) and motor skills domain suggests that the hcrt system may have a role in the motor skill performance of HIV-positive women. Further investigations need to be conducted to determine the role of the hcrt system in motor and cognitive performance of HIV-positive individual.

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HIV-1 TAT REGULATES DOPAMINE NEUROTRANMISSION. Miller, DR, BS 1, Streit, S, BS 1, Saha, K, Ph.D. 1, Koutzoumis, D, BS 1, McLaughlin, JP, Ph.D. 1, Streit, WJ, Ph.D. 1, Khoshbouei, H 1; 1Department of Neuroscience, University of Florida, Gainesville, FL 32608.

HIV-associated neurocognitive decline is a significant public health. The HIV-1 Tat protein is implicated in the onset and progression of HIV-associated neurocognitive decline (HAND). Recent work has shown HIV-1 Tat potentiates the effects of psychostimulants such as methamphetamine and cocaine. Psychostimulants target dopamine (DA) neurotransmission and alter the activity of DA transporter (DAT), the main regulator of DA transmission in the brain. In this study we investigated HIV-1 Tat regulation of tyrosine hydroxylase and DAT in the midbrain and striatum of wild-type and GT-tg bigenic mice which utilize a Tet-On promoter to produce dose-dependent HIV-1 Tat levels in astrocytes. Doxycycline (Dox) treated GT-tg mice exhibited increases in DAT expression, even after Dox is no longer administered as compared to wild-type or GT-tg controls mice treated with Dox or saline, respectively. We also examined the influence of HIV-Tat on DAT-induced DA efflux and DAT trafficking before and after exposure to methamphetamine. Since microglia are a primary target of HIV-1 Tat within the brain once it has crossed the blood-brain barrier, we investigated the interaction between dopaminergic neurons and microglia by examining the nature and mode of contacts between microglia and dopaminergic neurons in terms of number of contacts and the presence or absence of physical contact, as well as the ratio of microglia per dopaminergic neurons in multiple brain regions. Our preliminary data suggest that HIV-1 Tat may alter DA systems by a multitude of direct and indirect mechanisms.

MELATONIN, BUT NOT NOVEL MELATONIN RECEPTOR AGONISTS NEU-P11 AND NEU-P67, DISPLAYS ANTICONVULSANT ACTIVITY IN MICE. Mosinska Paula, PM, MS 1, Socala , KS 2, Nieoczym , DN 2, Fichna , JF, Ph.D. 1, Wlaz , PW, Ph.D. 2; 1Department of Biochemistry, Medical University of Lodz, Lodz, 90-4192Institute of Biology and Biochemistry, Maria Curie-Skłodowska University, Lublin, 20-033.

Background: Melatonin (MLT) is known to exhibit neuroprotective effects in the in vivo models of neurodegenerative diseases through a mechanism that implicates melatonin receptors (MT). However, the use of MLT is limited due to its short half-life, thus novel compounds are of particular need. The aim of this study was to compare the anticonvulsant properties of novel MT agonists Neu-P11 and Neu-P67 with MLT in mice. Methods: The anticonvulsant activity of tested compounds was evaluated in pentylenetetrazole- (PTZ) and electrically-induced convulsions. The effect of studied compounds on skeletal muscular strength in mice was quantified in the grip test. Furthermore, locomotor activity after administration of the tested compounds was assessed. Results: In the MEST and 6-Hz tests, only MLT (50

and 100 mg/kg, i.p.) significantly increased the seizure threshold. The i.p. administration of MLT (100 mg/kg) and Neu-P67 (200 mg/kg) resulted in significantly elevated PTZ seizure threshold for forelimbs tonus. The compounds did not affect muscle strength.The locomotor activity after administration of all tested compounds was significantly decreased. Conclusion: Our study confirms the anticonvulsant potency of MLT and shows that novel synthetic MT agonists Neu-P11 and Neu-P67 have no effect on epileptic seizures in mice. Our data suggest that the activation of MT can be used in the treatment of seizures, but further pharmacological characterization is needed to understand the anticonvulsant activityof MLT and to design efficient MT-targeting antiepileptic drugs.

MANIPULATION OF SYNAPTIC MICRORNAS IN-VIVO REDUCES ALCOHOL CONSUMPTION BY REVERSING THE EFFECTS OF ALCOHOL ON SYNAPTIC MRNA LEVELS. Most, D., BS 1, Black, M., BS 1, Blednov, Y.A., Ph.D. 1, Mayfield, R.D., Ph.D. 1, Harris, R.A., Ph.D. 1; 1The Waggoner Center for Alcohol and Addiction Research, The University of Texas at Austin, Austin, TX 78712.

Local translation of synaptic mRNAs plays a major role in synaptic structure and function. We previously showed that chronic alcohol consumption perturbs a large network of co-expressed synaptic mRNAs, which may explain the persistent remodeling of synaptic structures seen in the alcoholic brain. MicroRNAs can regulate many mRNAs at once, and are also altered in response to alcohol consumption, highlighting the potential role of microRNAs as regulators of mRNA expression (Most et al. 2014). Here we study whether the manipulation of microRNAs in-vivo affects alcohol consumption and preference by altering the expression of known alcohol-responsive microRNAs. Mice were cannulated in the prefrontal cortex, and introduced with 15% alcohol solution for 3-4 weeks. Five microRNAs were manipulated using microRNA mimics, antagomiRs and a cocktail of antagomiRs. Changes in alcohol consumption and preference in response to treatment were tested. AntagomiR-411 was found to significantly reduce alcohol consumption and preference for at least a week. Results from elevated plus maze and open field tests show that the reduction is not caused by treatment-induced anxiolysis. We validate the cannulation sites and the treatment-induced changes in microRNA expression levels, as well as changes in expression of predicted GABA, glutamate and neuroimmune mRNAs and proteins. In summary, chronic alcohol consumption was found to change the expression of a synaptic microRNA. Manipulation of that microRNA was found to change alcohol consumption levels, underscoring the essential role of microRNAs.

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SELECTION OF GRNAS TO TARGET THE HIV-1 QUASISPECIES WITH CRISPR/CAS9. Nonnemacher, M.R., Ph.D. [1], Dampier, W, Ph.D. [1], DeSimone, M, MS [3], Pirrone, V, Ph.D. [1], Kercher, K, MS [2], Passic, S, MS [2], Williams, J, BS [2], Wigdahl, B, Ph.D. [1]; [1]Department of Microbiology and Immunology, Drexel University College of Medicine, Philadelphia, PA 19102[2]Center for Molecular Virology and Translational Neuroscience, Institute for Molecular Medicine and Infectious Disease, Drexel University College of Medicine, Philadelphia, PA 19102[3]School of Biomedical Engineering and Health Systems, Drexel University, Philadelphia, PA 19104.

HIV-1 viral persistence during long-term antiretroviral therapy is a major hurdle to a cure. Genomic editing techniques, like the CRISPR/Cas9 system, hold promise to permanently excise integrated virus from a host cell. Targets are defined by a 20 nucleotide guide RNA (gRNA) complementary to the desired genomic region. However, due to the rapid mutation rate intrinsic to HIV-1 replication, the virus in patients exists as a collection of distinct genomic variants, termed quasispecies. Presented here is a methodology for designing gRNA sequences to cleave a patient's HIV-1 quasispecies. PBMC genomic DNA was isolated from patients in the Drexel Medicine CNS AIDS Research and Eradication Study (CARES) Cohort as well as from brain and spleen tissue from the National NeuroAIDS Tissue Consortium (NNTC) and the long terminal repeat (LTR) of the HIV-1 quasispecies was sampled using Next Generation Sequencing (NGS). gRNAs were computationally selected by examining their binding potential across a random training set of CARES patient samples. A package of 4 or 10 gRNAs were selected based on the training set which cleaved the entire detectable quasispecies of the remaining CARES samples and of the NNTC samples an average of 3.4+/-1.7 or 5.4+/-3 times, respectively. The package was further tested against a national sampling of subtype B North American LTRs from the Los Alamos National Database and was shown to cleave all sequences. This work presents a step towards understanding the complex task of using excision therapy to target HIV-1 quasispecies in the infected patient population.

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ASTROCYTE AEG-1 REGULATES ER STRESS RESPONSES IN THE CONTEXT OF HAND. Nooka, S, MS 1, Ghorpade, A, Ph.D. 1; 1Cell Biology and Immunology, University of North Texas and Health Science Center, Fort Worth, TX 76107.

Endoplasmic reticulum (ER) stress has recently been linked to neurological disorders, including HIV-associated neurocognitive disorders (HAND). We recently showed astrocyte elevated gene (AEG)-1, a multifunctional oncogene regulating astrocyte migration, proliferation and neuroinflammation. AEG-1 upregulation in Huntington's disease model suggests its role in ER stress responses in aging and HAND. However, its involvement in ER stress responses during HIV-1 infection is not known. In this study, we investigated HIV-1 and anti-retroviral therapy (ART) drugs mediated ER stress i.e., unfolded protein response (UPR) pathway activation, and astrocyte AEG-1 expression, intracellular localization during ER stress. RT-PCR and western blot analysis revealed that HIV-1, IL-1β and ART drugs activated UPR pathway and autophagy in astrocytes. Moreover, astrocytes exposed to ER stress compounds upregulated AEG-1 expression. Confocal analysis and mPTP assay showed AEG-1 colocalization with calnexin and mitochondrial damage with ER stress. In addition, AEG-1 overexpression upregulated ER stress markers such as BiP, PERK, and CHOP that were further enhanced by IL-1β treatment. Immunocytochemical studies also showed increased autophagy markers i.e., LC3 and P62 in AEG-1 overexpressing astrocytes. In summary, our study highlights that HIV-infection and ART drugs induce ER stress in astrocytes that is further exacerbated by AEG-1. Therefore, elucidation of AEG-1 regulated UPR pathway could assist in targeting astrocyte-induced ER stress responses in HAND.

LONG TERM ADMINISTRATION OF METHAMPHETAMINE IN TAT MICE CAUSES ALTERATION OF BEHAVIOR AND NEUROPLASTICITY GENE EXPRESSION – IMPLICATIONS IN NEUROAIDS. Nookala,

Anantha Ram, BS 1, Kumar, Anil, Ph.D. 1; 1Department of Pharmacology & Toxicology, University of Missouri Kansas City, Kansas city, MO 64108.

Methamphetamine (MA) abuse is common among individuals infected with HIV-1. These HIV-1 infected individual's exhibit greater neuronal injury and higher cognitive decline. Several HIV-1 proteins, specifically gp120 and HIV-1 Tat have been shown to affect neurocognition. However, combined effect of MA and HIV-1 Tat on the alteration of behavior and neuroplasticity gene expression is not well documented. The present study was undertaken to determine the combined effect of MA and HIV-1 Tat on behavior and neuroplasticity gene expression. Doxycycline (DOX)-inducible HIV-1 Tat (1-86) transgenic mice were administered with 6mg/kg MA twice a day and behavior studies were performed in both sexes. As measured by open field assay, the ambulatory activity was decreased in Tat +ve mice. However, administration of MA increased the ambulatory activity in both Tat –ve mice and Tat +ve mice. MA administered mice have showed decreased anxiety levels as assessed by the time spent in the light compartment during Light/dark box assay. In water maze task, there was a significant increase in escape latency in all the groups compared to control mice, particularly more in Tat transgenic mice that were administered MA. Furthermore, we observed a significant decrease in the protein expression of CNTF, Lif in entorhinal cortex and prefrontal cortex. Furthermore, we observed decrease in mRNA expression of CNTF, IL-11 and BDNF in parietal cortex. This study therefore provides novel insights into the dysregulated expression of various neuroplasticity genes in different brain regions.

Supported by NIDA/NIH.

VEGF ALTERATIONS INDUCED BY ANTIDEPRESSANT DRUGS IN FEMALE RATS UNDER CHRONIC STRESS CONDITION. Nowacka-Chmielewska, M, Ph.D. 1, Paul-Samojedny , M, Ph.D. 2, Bielecka, AM, Ph.D. 4, Obuchowicz, A, Ph.D. 4; 1Laboratory of Molecular Biology/Faculty of Physiotherapy, The Jerzy Kukuczka Academy of Physical Education in Katowice, Katowice, 40-0652Center For Experimental Medicine, Medical University of Silesia, Katowice, 40-7523Department of Medical Genetics, Medical University of Silesia, Sosnowiec, 41-2004Department of Pharmacology, Medical University of Silesia, Katowice, 40-752.

Vascular endothelial growth factor (VEGF) is believed to play a role in neurogenesis and response to stress. Although a definite link between the action of antidepressants and VEGF has not been elucidated yet, it is assumed that VEGF is important for the appearance of antidepressive effect. Therefore, the aim of the present study was to estimate the effect of desipramine (10mg/kg), fluoxetine (5 mg/kg) or tianeptine (10 mg/kg) given chronically on the number of VEGF mRNA copies in the brain structures of female rats subjected to chronic social instability stress. Furthermore, the alterations in serum VEGF concentration elicited by chronic stress and antidepressants were evaluated. Hormonal markers of stress were measured in parallel to behavioural tests. The relative adrenal weight, plasma corticosterone and ACTH concentrations were elevated in the stressed rats. Moreover, the exposure to chronic stress increased sucrose preference and diminished general locomotor activity. The exposure to stress has elevated VEGF expression in all studied structures: hippocampus, amygdala and hypothalamus. This effect of stress on VEGF mRNA level in the amygdala and hypothalamus was attenuated predominantly by desipramine. However, we have also noted a decrease in VEGF concentration in the serum of stressed rats. This decline was not reversed by the treatment with antidepressants. The presented data suggest that under stress conditions, VEGF may play a particular

role in the mechanism of action of antidepressants, most probably by modulating the activity of noradrenergic system.

EXAMINATION OF ILLICIT DRUG 5-METHOXY-N,N-DIISOPROPYLTRYPTAMINE, 5-MEO-DIPT ACTIONS IN THE RAT BRAIN. Noworyta-Sokołowska, K., MS 1, Gołembiowska, K., Ph.D. 1; 1Department of Pharmacology, Institute of Pharmacology, Polish Academy of Sciences, Kraków, 31-343.

5-Methoxy-N,N-diisoptopyltryptamine (5-MeO-DIPT) is the most popular designer tryptamine. In vitro data showed that 5-MeO-DIPT act as potent serotonin transporter (SERT) inhibitor and serotonin 5-HT 1A and 5-HT 2A receptor agonist. However, its neuronal mechanism of actions is not clear. Therefore, we tried to investigate effect of 5-MeO-DIPT on monoamine and amino acid neurotransmitters in the rat brain. 5-MeO-DIPT was given subcutaneously at doses of 5, 10 or 20 mg/kg. Determination of extracellular levels of dopamine (DA), serotonin (5-HT), γ-aminobutyric acid (GABA) and glutamate (Glu) in the striatum (Str), frontal cortex (FCx) and Nucleus accumbens (NAc) were carried out using microdialysis in freely moving rats. The obtained samples were analyzed by HPLC with electrochemical detection. 5-MeO-DIPT increased extracellular level of DA, 5-HT, GABA and Glu in all studied brain regions. These data indicate that 5-MeO-DIPT by enhancing extracellular level of 5-HT via inhibition of SERT indirectly or directly influences DA, GABA and Glu release. Observed changes may underline hallucinogenic properties of 5-MeO-DIPT. Karolina Noworyta-Sokołowska is a holder of scholarship from the KNOW sponsored by Ministry of Science and Higher Education, Republic of Poland.

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SEX DIFFERENCES IN THE EFFECTS OF ACUTE PERIPHERAL IL-1BETA ADMINISTRATION ON THE BRAIN AND SERUM VEGF EXPRESSION IN RATS . Obuchowicz, E, MD, M.Ph. 1, Nowacka, MM, Ph.D. 2, Paul-Samojedny , M, Ph.D. 3, Bielecka-Wajdman , AM, Ph.D. 1; 1Department of Pharmacology, School of Medicine in Katowice , Medical University of Sielsia, Katowice, 40-7522Laboratory of Molecular Biology, Faculty of Physiotherapy, The Jerzy Kukuczka Academy of Physical Education, Katowice, 40-0653Department of Medical Genetics, Faculty of Pharmacy with Division of Laboratory Medicine, Medical University of Silesia, Sosnowiec, 41-200.

Sex differences in brain morphology and function, and dimorphism of immunity are believed to be the basis of differences in the prevalence and course of neuropsychiatric diseases between women and men (Bao and Swaab, Front Neuroendocrinol, 2011). In this study, the differences in VEGF system under normal conditions and in response to recombinant rat IL-1beta/IL-1F2 (50 microg/kg, R&D Systems) given ip. were estimated in male and female rats. Females were in the estrus phase. The VEGF levels were determined by ELISA (R&D Systems) and VEGF mRNA by qRT-PCR assay. In females, the pituitary and serum VEGF levels were higher than in males, and a tendency towards an increased number of VEGF mRNA copies was noted in the hypothalamus, pituitary and hippocampus. Four hours after IL-1beta injection, a higher serum IL-1beta level was detected in females than in males. In male rats, IL-1beta decreased VEGF mRNA in the amygdala. In females, IL-1beta challenge increased the pituitary VEGF mRNA and serum VEGF levels but reduced the pituitary VEGF concentration. A negative correlation

between the serum VEGF levels and its concentration in the pituitary gland was found. These results suggest the significance of VEGF as a link between the brain and periphery in females under inflammatory condition and point to the need to consider sex-related differences when drawing conclusions about VEGF alterations during inflammation.

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ALCOHOL AND HIV-1 DIFFERENTIALLY REGULATE TOLL LIKE RECEPTOR (TLRS) EXPRESSION AND SIGNALING IN PRIMARY HUMAN ASTROCYTES. Pandey, R, Ph.D. 1, Gorpade, A, Ph.D. 1; 1Cell Biology and Immunology, University of North Texas and Health Science Center, Fort Worth, TX 76107.

About 69% of human immunodeficiency virus-1 (HIV-1)-positive individuals exhibit some form of HIV-associated neurocognitive disorders (HAND). Several studies have reported that HIV-1 virions, viral proteins and alcohol, individually have direct or indirect effects on HAND pathophysiology. Recently, we showed that alcohol activates astrocytes and regulates inflammation via cPLA2 in HAND. Toll-like receptors (TLRs) are a family of innate immune system receptors that respond to pathogen-derived and tissue damage-related ligands. TLR signaling in immune cells, astrocytes, microglia and neurons may play roles in the pathogenesis of multiple diseases. TLRs are a “missing” link in alcohol-mediated astrocytic response in context of HAND since TLR stimulation by alcohol in glial cells induces secretion of pro-inflammatory molecules. Thus, we explored the role of TLRs in alcohol-induced inflammation and cytotoxicity in primary human astrocytes with HAND. TLRs signaling gene array was performed to screen altered profiles for all 10 TLR family members and 74 downstream signaling molecules. Ingenuity pathway analysis (IPA) was performed to identify potential signaling nodes. Data suggested that HIV-1 and/or EtOH led to differential TLRs expression in astrocytes. We confirmed all 10 TLRs by real-time PCR in four independent astrocyte donors. Alcohol alone and with HIV-1, significantly upregulated TLR1, 2, 3, 4, 5 and 9 as compared to controls and HIV-1 alone. We propose that TLRs regulation plays an important role in astrocytes inflammation upon HIV-1 and EtOH exposure.

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GALECTIN-1 REDUCES NEUROINFLAMMATION VIA MODULATION OF THE NITRIC OXIDE-ARGINASE NETWORK IN MICROGLIA: IMPLICATIONS FOR A NEUROPROTECTIVE ROLE IN HIV ASSOCIATED NEUROCOGNITIVE DISORDERS. Parikh, NU, MD 1, Magnum, C 1, Aalinkeel, R, Ph.D. 1, Reynolds, JL, Ph.D. 1, Sykes, DE, Ph.D. 1, Mammen, M, MD 1, Schwartz, SA, MD, Ph.D. 1, Mahajan, SD, Ph.D. 1; 1Department of Medicine, Division of Allergy, Immunology & Rheumatology, State University of New York at Buffalo, Clinical Translational Research Center, Buffalo, NY 14226.

Activation of microglia in HIV infected patients results in neuroinflammation contributing to the development of HIV associated neurocognitive disorders (HAND). Nitric oxide (NO) plays an important role in neuroinflammation. L-arginine (ARG) is used as a substrate by both nitric oxide synthase (NOS) and arginase to produce nitric oxide (NO) and urea. Increased arginase activity results in depleting ARG in microglia via reducing the available arginine substrate for NO production. We hypothesize that Galectin-1 decreases NO production via the increased arginase I expression and augments expression of the cationic amino acid transporter 1 (CAT-1) resulting in decreased nNOS activity in microglia thereby

reducing neuroinflammation. Cytokine stimulated HIV transfected human microglial cells CHME-5/HIV were treated with Galectin-1 (1mM) and changes in oxidative stress were evaluated by measuring ROS and NO production and expression levels of Arginase I, CAT-1. Our results show that cytokines increased oxidative stress in CHME-5/HIV by 53% ($p<0.01$), while treatment with Galectin-1 (1mM) reduced oxidative stress by 61% ($p<0.01$). Microglia treated with Galectin-1 showed a 50% ($p<0.01$) and 83% ($p<0.001$) decrease in NO production and nNOS gene expression respectively, while there was a 4.6 fold ($p<0.01$) and a 43% ($p<0.05$) increase in the arginase I activity and CAT-1 gene expression respectively. Our data suggests that Galectin-1 treatment could reduce neuroinflammation via the modulation of the nitric oxide-arginase network and play a neuroprotective role in HAND.

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EPIGENETIC MODULATIONS DUE TO CHRONIC ALCOHOL TREATMENT AND ITS ASSOCIATION WITH THE CANNABINERGIC PATHWAY . Parira, T, MS 1, Figueroa, G, BS 1, Casteleiro, G 1, Laverde, A 1, Yndart, A, BS 1, Muñoz, K, MS 1, Nair, M.P., Ph.D. 1, Agudelo, M, Ph.D. 1; 1Department of Immunology, Herbert Wertheim College of Medicine,Florida International University, Miami, FL 33199.

Chronic alcohol abuse is a complex disorder governed by several factors such as cannabinoid receptors and histone deacetylases (HDACs). Epigenetic studies play a major role in understanding the effects of substances of abuse. Our previous studies, along with others, have shown modulation of HDACs and histone acetylation due to acute alcohol treatments in brain cells and tissues. In lieu of previous research, to further understand the epigenetic changes due to alcohol on the periphery, this study aims to elucidate the epigenetic modulations induced by chronic alcohol treatment (CAT) on monocyte derived dendritic cells (MDDCs) and its association with the cannabinergic pathway. Experiments were conducted with MDDCs after treatment with alcohol (0.2%) for 5 days with or without cannabinoids, JWH-015 (CB2 agonist) and AM-630 (CB2 antagonist). Interestingly, our results show that CAT reduced HDAC1, 2 and CB2 gene expression while modulating histone H4 levels and inducing H4K12ac modification, possibly through cannabinergic mechanisms as demonstrated by the antagonistic effect of AM-630. Furthermore, an increase in cytokine production by CAT was observed and this effect was blocked by the synthetic cannabinoids. In summation, our results show that cannabinoids are modulating alcohol induced epigenetic effects on MDDCs; therefore, the use of synthetic cannabinoids may be of therapeutic significance for the treatment of alcohol use disorders.

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METHAMPHETAMINE-INDUCED ABERRANT NEUROGENESIS: PROTECTION BY EXERCISE. Park, MS, Ph.D. 1, Skowronska, M, Ph.D. 1, Levine, H 1, Toborek, M, MD, Ph.D. 1; 1Department of Biochemistry and Molecular Biology, University of Miami School of Medicine, Miami, FL 33155.

HIV-infected methamphetamine (METH) abusers exhibit higher HIV viral loads and more severe neurological complications than non-abusers. We hypothesize that potentiation of HIV production in neural stem cells (NSCs) by METH can result in impaired neurogenesis. Indeed, pre-exposure to METH potentiated HIV replication in NSCs via activation of NF-κB and SP-1 and stimulation of HIV LTR. In addition, exposure to METH affected differentiation of NSC to mature neurons. While no effective

therapy is available for the treatment of METH-induced neurotoxicity, exercise is a highly promising approach to improve substance abuse outcomes. Therefore, we employed an animal model to evaluate the impact of METH and exercise on NSC differentiation. The study was based on a chronic exposure to METH (5 days with an escalating doses at 3 h intervals), followed by two weeks of exercise. Control mice expressed strong immunoreactivity for doublecortin (DCX, a marker for immature neurons), which branched to the distal part of the dentate gyrus. In contrast, the processes of the DCX-positive cells were visibly underdeveloped and the number of DCX-positive cells decreased as the result of METH exposure. Importantly, voluntary exercise protected against this effect. Mechanistically, the beneficial impact of exercise was linked to antioxidative and anti-inflammatory effects. These results indicate that exercise can attenuate METH-induced aberrant neurogenesis and suggest that physical activity could also be beneficial to counteract METH- induced progression of HIV-associated neurodegeneration.

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EFFECTS OF DRUGS OF ABUSE ON INTERACTIONS BETWEEN HIV-INFECTED MACROPHAGES AND NEURONS: IMPLICATIONS WITH HAND IN DRUG ABUSERS. Patters, BJ, BS 1, Sinha, N, BS 1, Kumar, S, Ph.D. 1; 1Department of Pharmaceutical Sciences, College of Pharmacy, University of Tennessee Health Science Center, Memphis, TN 38163.

In infected individuals, HIV enters the CNS, causing neuroinflammation and neurotoxicity that results in a wide range of neurocognitive symptoms collectively referred to as HIV-associated neurocognitive disorders (HAND). Tobacco and alcohol consumption, behaviors common in the HIV+ population, have been shown to increase viral load and HIV pathogenesis. Ethanol consumption has also been shown to increase the severity of HAND. While the mechanisms by which these drugs of abuse (DoA) affect HIV-induced neurotoxicity are not fully understood, HIV-infected macrophages likely play a significant role. We hypothesize that chronic exposure of macrophages to DoA induces viral replication and production of oxidative stress through cytochrome P450 (CYP) pathways. This may lead to production of viral particles, reactive oxygen species (ROS), and other soluble factors that further contribute to neuronal death. To test this, we treated SH-SY5Y neuronal cells with ethanol, tobacco constituents, and media supernatant from HIV+ macrophages (derived from U1 monocytes), which independently caused toxicity. The results showed that media derived from DoA-exposed HIV+ (U1) and HIV- (U937) macrophages, when applied to neurons, caused altered expression of a number of genes related to the cytochrome P450 (CYP) and oxidative stress pathways. These results suggest that exposure of HIV+ macrophages to DoA causes neuronal damage through CYP- and oxidative stress pathways. Examination of the underlying mechanisms of DoA- and HIV-mediated neuronal damage, likely through those pathways, is underway.

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PARP INHIBITION IN LEUKOCYTES DIMINISHES INFLAMMATION VIA EFFECTS ON INTEGRINS/CYTOSKELETON AND PROTECTS THE BLOOD BRAIN BARRIER. Persidsky, Y, MD, Ph.D. 1, Reichenbach, N, BS 1, Zuluaga-Ramirez, V 1, Dykstra, H, BS 1, Gajghate, S, MS 1, Rom, S, Ph.D. 1;

1Department of Pathology and Laboratory Medicine, Lewis Katz School of Medicine, Temple University, Philadelphia, PA 19140.

Neuroinflammation is characterized by leukocyte infiltration into the brain resulting in blood brain barrier (BBB) dysfunction and contributing to morbidity in multiple sclerosis, encephalitis, traumatic brain injury and stroke. Identification of pathways that decrease the inflammatory potential of leukocytes would prevent such injury. In this study, we explored the idea that selective inhibition of poly(ADP-ribose) type 1 (PARP-1) in leukocytes would diminish their engagement of brain endothelium. These effects are outside recognized of PARP-1 functions in DNA repair and transcriptional regulation. Indeed, PARP-1 suppression diminished leukocyte adhesion to and migration across BBB in vitro models and prevented BBB injury. In monocytes, PARP inactivation decreased conformational activation of integrins that plays a key role in their tissue infiltration. Such changes were mediated by suppression of activation of small Rho GTPases and cytoskeletal rearrangements in monocytes. In vitro observations were confirmed in vivo showing diminished leukocyte-endothelial interaction after selective PARP suppression in leukocytes and BBB protection. PARP knockout animals demonstrated substantial diminution of inflammatory responses in brain microvasculature and a decrease in BBB permeability. These results suggest PARP inhibition in leukocytes as a novel approach to BBB protection in the setting of endothelial dysfunction caused by inflammation-induced leukocyte engagement.

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SECOISOLARICIRESINOL DIGLUCOSIDE (SDG) PROTECTS BLOOD BRAIN BARRIER IN VITRO AND IN VIVO DURING NEUROINFLAMMATION . Persidsky, Y, MD, Ph.D. 1, Reichenbach, N, BS 1, Zuluaga-Ramirez, V 1, Rom, S, Ph.D. 1, Jordan-Sciutto, K, Ph.D. 2; 1Department of Pathology and Laboratory Medicine, Lewis Katz School of Medicine, Temple University, Philadelphia, PA 191402Department of Pathology, School of Dental Medicine University of Pennsylvania, Philadelphia, PA 19147.

HIV-associated neurocognitive disorder (HAND) affects approximately half of HIV-positive patients despite the success of antiretroviral therapy in suppressing viral replication. HAND persistence may be related blood brain barrier (BBB) dysfunction, low levels of viral replication, neuroinflammation and oxidative stress. We demonstrated BBB damage and neuroinflammation in the CNS of patients with HAND. Here, we evaluated the potential of SDG (a flaxseed lignin), an agent with known antioxidant and anti-inflammatory properties, to reduce BBB dysfuction and neuroinflammation. SDG treatment attenuated monocyte adhesion to (80%) and migration across primary human brain microvascular endothelial cells, BMVEC (30-46%) using in vitro BBB models. SDG treatment of TNFα-/IL1β activated BMVEC attenuated increases in VCAM-1 expression. Human monocyte stimulation with consensus sequence mimicking monocyte cytoskeleton interactions with adhesion molecules led to a 1.8 fold increase in fibrillary/globular actin ratio, a change seen in actin during inflammation, these skeletal changes were completely blocked by SDG. CCL2 stimulation of monocytes led to 26-fold increase in active VLA-4 (associated with enhanced monocyte adhesion and migration), which was attenuated by 33% with SDG. Using mouse model of encephalitis/meningitis (intracerebral injection of TNFα) we demonstrated that oral SDG pretreatment for 4 h led to 55-60% decrease in adhesion to and migration across BBB. In summary, SDG emerged as promising new agent attenuating inflammatory responses at BBB.

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DOPAMINE AFFECTS MIGRATION AND MORPHOLOGY OF HUMAN NEUTROPHILS THROUGH D1-LIKE DOPAMINERGIC RECEPTORS. Pinoli, M, BS 1, Rasini, E, BS 1, Legnaro, M, BS 1, De Eguileor, M, BS 2, Pulze, L, Ph.D. 2, Cosentino, M, MD, Ph.D. 1, Marino, F, BS 1; 1Center of Research in Medical Pharmacology, University of Insubria, Varese, 211002Department of Biotechnology and Life Sciences, University of Insubria, Varese, 21100.

Dopamine (DA) affects the immune response, but little is known about DAergic modulation of innate immunity. Polymorphonuclear leukocytes (PMN) are innate immune cells that during inflammation migrate into peripheral tissues. Dopaminergic receptors (DR) were analyzed by Real Time PCR and flow cytometry. Migration was assessed by the Boyden chamber assay. Morphology was evaluated by transmission electron microscopy (TEM). Expression of the adhesion molecules CD11b and CD43 was analyzed by flow cytometry. DR mRNA were D4>D5>D1=D3>>D2. PMN were 82-89% and 18-58% positive, respectively, for D1-like (D1, D5) and D2-like (D2, D3, D4) DR. Migration induced by fMLP 0.1 µM was (mean±SEM) 9.4±1.2 µm. DA 1 µM reduced fMLP-induced migration to 26.2±18.0% (n = 5, $P<0.01$ vs fMLP). The effect was reverted by the D1-like DR antagonist SCH-23390 1 µM (77.3±13.9%, n=3, $P>0.05$ vs fMLP and $P<0.05$ vs fMLP+DA) and mimicked by the D1-like DR agonist SKF-38393 0.1 µM (21.4±7.0%, n=4, $P<0.01$ vs fMLP). D2-like DR ligands did not affect migration. DA prevented fMLP-induced morphological changes of PMN, and was antagonized by SCH-23390. Preliminary experiments showed that DA 1 nM reduced fMLP-induced CD11b expression from 14369.3±3151.3 to 10219.7±1093.5 (n=3, P=0.08). Further experiments on CD11b as well as on CD43 expression are presently ongoing. Human PMN express all DR. D1-like DR inhibit fMLP-induced migration, and DA may also affect adhesion molecule expression. Clarifying the role of dopaminergic pathways in PMN might provide novel clues for the use of DAergic drugs as immunomodulating agents.

THE ACTIVATION OF PRO- AND ANTINOCICEPTIVE PENK-DERIVED PEPTIDES AS AN IMPORTANT ELEMENT IN PATHOLOGY OF NEUROPATHIC PAIN – BEHAVIOURAL STUDIES. Piotrowska, Anna 1, Starnowska, Joanna 1, Makuch, Wioletta 1, Mika , Joanna 1, Przewłocka, Barbara 1; 1Department of Pain Pharmacology, Institute of Pharmacology, Polish Academy of Sciences, Kraków, 31-343.

Proenkephalin (PENK) system was found to be represented in the regions involved in the nociception and is implicated in chronic pain. Met- and Leu-enkephalin are the major antinociceptive peptides arising from the enzymatic cleavage of proenkephalin. However, in addition to those, many other peptides are created, both opioid and non-opioid. The aim of the study was to analyze changes in level of pro- and antinociceptive peptides generated from PENK prohormone under neuropathic pain. Research was carried out on Wistar rats implanted with intrathecal (i.t.) catheters. Neuropathic pain was developed using Bennett's model (chronic constriction injury, CCI). Peptides: Met-enkephalin, Peptide E, Pro-peptides PENK(196-207;17-227;239-260) were dissolved in water and administered at single doses:1, 10 or 50 µg/5µl on day 7 after CCI. Two behavioral tests were conducted to measure allodynia (von Frey test) and hyperalgesia (cold plate test). Behavioral studies have shown that i.t. administration of peptides increased or diminished the allodynia and hyperalgesia on day 7 after CCI, depending on the dose. This shows that PENK-derived peptides may perform in central nervous system opposite actions,

both pro- and antinociceptive. The study allowed us to demonstrate the important contribution of pro-nociceptive peptides generated from PENK in the development of neuropathic pain. Identification of a bidirectional action of opioid systems in neuropatic pain are of importance to understanding this pathology and allow for the future design of a new strategy in the treatment of neuropathy.

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IMIPRAMINE ENHANCES EXPRESSION OF SOME NEURONAL MARKERS IN GLIA CELLS: A STUDY ON THE RAT PRIMARY MIXED GLIAL CULTURE. Plato, M.M., Ph.D. 1, Bielecka-Wajdman, A.M., Ph.D. 1, Orchel, J.J., Ph.D. 2, Obuchowicz, E.M., MD, M.Ph. 1; 1Department of Pharmacology, School of Medicine in Katowice, Medical University of Silesia, Katowice, 40-0552Department of Molecular Biology, Faculty of Pharmacy in Sosnowiec, Medical University of Silesia, Katowice, 40-055.

Imipramine is the oldest tricyclic antidepressant, recommended for use as a second-choice drug for endogenous depression and frequently applied in pharmacological studies as a reference antidepressant. Recently, we have shown that imipramine induced transformation of microglial cells in the rat primary mixed glial culture into cells with neuron-like phenotype (Obuchowicz et al., Pharmacol Rep 2014). The aim of this study was to check the effect of imipramine on the expression of some neuronal markers in the primary mixed glial culture. The cultures were prepared from the cerebral cortices of newborn Wistar rats and were incubated in standard conditions (37 0 C, 95% air, 5% CO2). On day 13, the culture medium was replaced with a medium containing imipramine (10 μM). After 6, 12, 24, 48, 72 h or 7 days of incubation with imipramine total RNA was extracted from cells using Trizol reagent (Invitrogen) according to the Chomczynski's method. The expression of nestin, β- tubulin, enolase 2, neurofilament (NF-200) and synaptophysine was analyzed by qRT-PCR assay (LightCykler 480, Roche) against a constitutive expression level of the housekeeping gene of glyceraldehyde 3-phosphate dehydrogenase. Imipramine increased mRNA expression of all studied neuronal markers in cell extracts prepared from the cultures exposed to the drug, especially for 24 – 48 h. It can be concluded that in in vitro conditions, imipramine induces transformation of microglia cells and influences expression of neuronal markers. These data confirm that the microglial cells are an important target for imipramine.

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ENHANCEMENT OF INTERLEUKIN-10 RESPONSE AND ANXIOLYTIC ACTIVITY FOLLOWING INTRASEPTAL NMDA GLUTAMATE RECEPTOR AGONIST INJECTION ARE MORE PRONOUNCED IN HIGH RESPONDER RATS . Podlacha, M. 1, Wrona , D. 1; 1Department of Animal and Human Physiology, University of Gdansk, Gdansk, 80-309.

Glutamate receptors have regulatory functions not only in the nervous system, but also in immunocompetent cells and anxiolytic effects. However, role of the medial septal (MS) NMDA receptors in modulation of interleukin-10 (IL-10) response and anxiolytic behavior in rats differing in stress susceptibility, remains unclear. Male Wistar rats (n=26) prior categorized as HRs (n=12) or LRs (n=14) in the novelty test (2 h) were exposed to the elevated plus-maze (EPM) test (5 min) in the baseline, 15 min (short period-SP) and 60 min (long period-LP) after injection of NMDA (n=14) at a dose of 0.25 μg/rat or

saline (0.5 µl/rat, n=12) via implanted cannulae into the MS. Plasma concentration of IL-10 (ELISA) was measured at the baseline and in the LP after the NMDA injection. In the LP after the NMDA injection, a significant increase in the IL-10 concentration (HRs: 356±18 pg/ml; LRs: 291±8 pg/ml) in comparison with the baseline (HRs: 118±12 pg/ml, p≤0.001; LRs: 116±15 pg/ml, p≤0.01) and the SAL (HRs: 184±11 pg/ml, LRs: 138±16 pg/ml; p≤0.05), more pronounced in the HRs, was observed (p≤0.05). Moreover, there was a significant (p≤0.001) increase in time spent in open arms in the HRs (SP: 31±10 s, LP: 59±8 s) as compared to the baseline value (20±6s, SP: p≤0.01, LP: p≤0.001) and SAL (20±8 s, p≤0.001) in the LP. These data indicate that the MS NMDA glutamate receptor activation under stress conditions significantly increases such a peripheral anti-inflammatory response as IL-10 level and anxiolytic activity in particular, in rats with higher behavioral activity and decreased anxiety.

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URMC-099 TRANSFORMATION OF THE EXPRESSION AND LOCALIZATION OF TRANSCRIPTION FACTOR-EB PROVIDES A POTENTIAL MECHANISM FOR RAB ENDOSOMAL NANOFORMULATED ANTIRETROVIRAL DEPOT FORMATION IN MONOCYTE-MACROPHAGES. Gnanadhas, Divya Prakash, Ph.D. 1, Dash, Prasanta K, Ph.D. 1, Lin, Zhiyi, MS 1, Puccini, Jenna M, Ph.D. 2, Gelbard, Harris A, MD, Ph.D. 2, Gendelman, Howard E, MD 1, Gorantla, Santhi, Ph.D. 1; 1Department of Pharmacology and Experimental Neuroscience, University of Nebraska Medical Center, Omaha, NE 681982School of Medicine and Dentistry, University of Rochester Medical Center, Rochester, NY 14642.

Long-acting nanoformulated antiretroviral therapy (nanoART) improves drug pharmacokinetics (PK) and biodistribution and limits systemic toxicities. Few significant obstacles of nanoART include drug retention, ease of administration and toxicity. URMC-099 has emerged as a candidate drug to overcome such limitations. Originally developed as a neuroprotective and anti-inflammatory agent, it was shown to affect nanoART PK by sequestering ART particles in early, late and recycling, Rab 5, 7 and 11 endosomes. This led to both reduced viral loads and CD4+ T lymphocyte protection in HIV infected humanized mouse model. To understand the underlying mechanisms, human monocyte-derived macrophages (MDM) were pretreated with nanoformulated atazanavir (nanoATV) and infected with ADA strain of HIV-1 with or without URMC-099. Western blot and confocal microscopy demonstrated that URMC-099 increases nuclear translocation of transcription factor-EB (TFEB). As TFEB is a master regulator of autophagy and lysosomal biogenesis, further analyses by western blot showed increased BECN1 (an autophagy marker) and decreased Rab 5 & 7 proteins (endosomal markers). Furthermore, increased viability of MDM in presence of URMC-099 confirms the involvement of autophagy and cellular clearance. Together, enhanced autophagy by URMC-099 is facilitating nanoART retention in virus-containing subcellular compartments by modulating cellular trafficking and enhance viral clearance. This finding opens up many therapeutic strategies of URMC-099 for HIV-1 infection and HIV Associated Neurocognitive Disorders.

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MUTATION OF HISTIDINE 547 ON THE HUMAN DOPAMINE TRANSPORTER ENHANCES DOPAMINE TRANSPORT AND ATTENUATES TAT-INDUCED INHIBITION OF DOPAMINE TRANSPORTER. Quizon, P.M. 1,

Sun, WL. 1, Yuan, Y. 2, Midde, N.M. 1, Huang, X. 2, Zhan, CG. 2, Zhu, J. 1; 1Department of Drug Discovery and Biomedical Sciences, South Carolina College of Pharmacy, University of South Carolina, Columbia, SC 292082Molecular Modeling and Biopharmaceutical Center, and Department of Pharmaceutical Sciences, College of Pharmacy, University of Kentucky, Lexington, KY 40506.

Abnormal dopaminergic transmission has been implicated as a risk determinant of HIV-1-associated neurocognitive disorders. HIV-1 Tat and cocaine synergistically increase synaptic dopamine (DA) levels by directly inhibiting DA transporter (DAT) activity, ultimately leading to dopaminergic neuron damage. Through integrated computational modeling prediction and experimental validation, we have identified key residues in DAT with which Tat interacts, which are critical for Tat-induced inhibition of DAT and transporter conformational transitions. This study investigated the functional influences of mutations of histidine547 (H547A) and its associated residues on human DAT in basal DA transport and Tat-induced inhibition of DA transport. Compared to wild type human DAT, H547A-hDAT displayed a 197% increase in the Vmax with no change in the Km. The increased Vmax in H547A was not accompanied by change in DAT surface expression. Results from other substitutions of His547 show that the Vmax was not altered in H547R but decreased by 99% in H547P and 60% in H547D, respectively. Importantly, H547 attenuated Tat-induced inhibition of DA transport observed in wild type hDAT. PMA, a PKC activator, produced a 40% inhibition of Vmax in wild type DAT and 61% in H547A, indicating a lower basal PKC activity in H547A. These findings demonstrate that His547 plays a crucial role in stabilizing basal DA transport and Tat-DAT interaction. This study provides mechanistic insights into identifying targets on DAT for Tat binding and improving DAT-mediated dysfunction of DA transmission.

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IDENTIFICATION OF COMMERCIALLY AVAILABLE ANALOGS OF DIALLYL SULFIDE FOR THEIR INCREASED INHIBITION OF CYP2E1 AND DECREASED CELLULAR TOXICITY IN MONOCYTES, ASTROCYTES AND HEPATOCYTES. Rahman, M A, MS 1, Rao, PSS, Ph.D. 1, Midde, N M, Ph.D. 1, Kumar, S, Ph.D. 1; 1Pharmaceutical Science, College of Pharmacy, The University of Tennessee Health Science Center, Memphis, TN 38163.

Diallyl Sulfide (DAS), a lipophilic thioether derived from garlic has shown to be protective against chemically induced hepatoxicity and alcohol & viral protein mediated cytotoxicity in HIV model systems. The effect is mainly due to the inhibition of CYP2E1-mediated metabolic activation of various chemicals. However, upon its metabolism by CYP2E1, DAS itself causes cellular toxicity. The aim of the current project is to find an analog of DAS, which is a stronger inhibitor but weaker substrate of CYP2E1. Four available analogs: Diallyl Ether (DE), Allyl Ethyl Sulfide (AES), Allyl Methyl Sulfide (AMS), and Thiophene (TP) were used for this study. Initially, these compounds were docked to the active site of CYP2E1, which showed that, in general, the binding capability of the analogs is decreased/unaltered compared to DAS. Using Vivid CY2E1 assay, we found that at 10µM concentration, DE inhibited CYP2E1 to slightly higher extent than the other compounds. Later, the analogs were tested for cytotoxicity upon treatment in U937 monocytes, SVGA astrocytes, and HepaRG hepatocyte. At 48 hour, cell viability was significantly higher in DE- and/or TP-treated cells than that of DAS in U937 & HepaRG cells. We observed that in DE- and TP-treated cells, caspase-3 activity were significantly lower than other compounds, suggesting that caspase-3 -mediated apoptosis is the likely mechanism of cell death. The analog with higher CYP2E1

inhibition and least cytotoxicity may further be used to develop novel compounds, which are expected to have clinical significance.

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EFFECT OF POLYARYL HYDROCARBONS (PAHS) OF CIGARETTE SMOKE ON HIV-1 REPLICATION IN MONOCYTIC CELLS: POSSIBLE ROLE OF CYTOCHROME P450 AND OXIDATIVE STRESS PATHWAY. Ranjit, Sabina 1, Mohammad, A Rahman 1, Patters, Benjamin 1, Midde, Narasimha M., Ph.D. 1, Sinha, Namita 1, Cory, Theodore J., Ph.D. 1, Rao, PSS, Ph.D. 1, Kumar, Santosh, Ph.D. 1; 1Pharmaceutical Sciences, University of Tennessee Health Science Center, Memphis, TN 38105.

Smoking aggravates HIV-1 pathogenesis and leads to decreased responses to antiretroviral therapy. However, the exact mechanism by which smoking enhances HIV-1 pathogenesis is unknown. Benzo(a)pyrene (BaP), Naphthalene (NPh), Phenanthrene (Phe), Benzo(a)antharacene (BeA) and Benzo(b)fluoranthene (BeF) are major carcinogens in cigarette. They require metabolic activation through cytochrome P450s (CYPs) to exert their toxic effects. We hypothesize that PAHs are metabolized by CYP-mediated pathway and produce reactive oxygen species (ROS). Oxidative stress resulting from increased ROS would aggravate HIV-1 replication. In the current study, we explored the acute (6-24 hours) and chronic (7 day) effect of BaP, NPh, Phe, BeA and BeF on CYPs and oxidative stress pathways in U937 monocyte cells. Only chronic treatment with BaP showed significant increase in the expression of CYPs (1A1, 3A4) and antioxidant enzymes (AOEs- SOD1 and catalase). As expected, we also observed increase in ROS and cell death. Next, we examined the chronic effect of BaP in U1 cells (HIV-1 infected monocytes). There was ~4 fold increase in HIV-1 replication with chronic BaP treatment. We also observed increase in the mRNA expression levels of CYPs but not AOEs. However, ROS levels decreased significantly, which may be attributed to immense cell death (~50%). Further, we will investigate the underlying mechanism for BaP-mediated HIV-1 replication in U1 cells. The outcomes from the present work are clinically relevant as they may help to determine optimal dosing regimens for HIV positive smokers.

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HIV-1 NEF EXPRESSION BY ASTROCYTES INDUCES GASTROINTESTINAL AND LUNG INFLAMMATION AND INCREASES IN CD68+ MACROPHAGES. Rivera, J, MS 1, Cruz, M, BS 1, Isidro, R, BS 1, Loucil, R, Ph.D. 1, Noel, R, Ph.D. 1, Rivera-Amill, V, Ph.D. 1; 1Ponce Research Institute, Ponce Health Science University, Ponce, PR 00732.

Patients diagnosed with human immunodeficiency virus (HIV) and treated with combined antiretroviral therapy (cART) can avoid progression to AIDS but remain susceptible to development of neurocognitive disorders along with systemic inflammation. CART targets viral replication, but early viral protein production is not inhibited. Nef is an early viral protein that is known to contribute to the disruption of many immunological responses. Previous work in our lab has demonstrated that focal production of Nef by astrocytes in the hippocampus causes systemic damage, including small Intestine (SI) and lung inflammation. Additionally, analysis of tissue demonstrated loss of villus morphology and

reduced surface area in this model. Lastly, in the same model, it has been demonstrated that in the presence of increased amounts of CCL2 there is a greater infiltration of peripheral macrophages. Given this, the purpose of the current study is to determine if the increased number of macrophages leads to an enhance inflammatory response in SI and lung tissue. Sprague Dawley rats were infused in the right hippocampus with astrocytes transfected to produce Nef or GFP (control). Two days after surgery, the rats were sacrificed and the lung and SI were collected. Lung and SI tissue were used for immunofluorescence of CD68. Analysis of CD68 showed an increase in SI and lung macrophages in Nef rats in comparison with GFP (Control). Our findings provide evidence that Nef expression in astrocytes causes a rapid increase in systemic inflammation that is mediated by macrophages.

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TETRAHYDROCANNABINOL (THC)-MEDIATED SUPPRESSION OF HUMAN PLASMACYTOID DENDRITIC CELL AND MONOCYTE MATURATION. Rizzo, M.D., BS [1], Henriquez, J.E., MS [1], Crawford, R.B., BS [1], Kaminski, N.E., Ph.D. [1]; [1]Institute for Integrative Toxicology, Michigan State University, East Lansing, MI 48824.

Tetrahydrocannabinol (THC), the major psychoactive component of marijuana, has been well characterized as an immunosuppressant in human and murine model systems and is controversial as a therapeutic due to its potential health risks. The upregulation of MHC II, CD83 and CD86 are key events during the maturation of plasmacytoid dendritic cells (pDC) and monocytes, and are critical for T cell activation and clonal expansion. Herein, we investigated the role of THC on human blood pDC and monocyte maturation by measuring HLA-DR (MHC II), CD83 and CD86 levels. In human PBMCs stimulated with CpG for 24 hours, the percentage of pDC and monocytes expressing CD83 and CD86 along with HLA-DR levels showed a concentration-dependent suppression with THC. To elucidate a potential mechanism of suppression of pDC and monocyte maturation by THC, the expression levels of the adaptor protein EAT-2 were explored. EAT-2, which is a member of the SLAM family of adaptor proteins, plays a crucial role in cytokine production and expression of costimulatory molecules. After CpG-induced activation for 24 hours, THC decreased the percentage of pDC and monocytes expressing EAT-2 in a concentration-dependent manner. Collectively, these findings demonstrate the suppressive effects of THC on the maturation of pDC and monocytes along with a potential mode of suppression (EAT-2). These effects may result in dysfunctional T cell activation and clonal expansion, which is of particular importance for immunocompromised portions of the human population (e.g. cancer and HIV patients).

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ROLE OF AUTOPHAGY ON HIV-INFECTED HUMAN ASTROCYTES FUNCTIONALITY . Rodriguez, M, Ph.D. 1, Lapierre, J, MS 1, Estrada, H, BS 1, Dever, S, Ph.D. 1, Madhavan , N, Ph.D. 1, El-Hage, N, Ph.D. 1; 1Department of Immunology, Florida International University College of Medicine, Miami, FL 33199.

Although it is well accepted that HIV-1 infiltrates the brain shortly after infection, the mechanisms that lead to HIV-1 latency in astrocytes are still not completely understood. Here we investigated possible mechanisms involving “latency” in HIV-1-infected astrocytes. We explored the effect of the

autophagy inducer rapamycin, as well as silencing with siRNA against the ATG6 and ATG5 autophagy-related genes on HIV-infection and functionality of astrocytes. Since HIV-1 and opioids are interlinked epidemics that exacerbate HIV neuropathology, we investigated the role of autophagy as a mechanism of morphine-induced effects. We demonstrate that rapamycin re-activates HIV-p24 production and increases the release of calcium and inflammatory cytokines. Exposure with morphine did not alter viral p24 production; however, in combination with rapamycin, the functionality of HIV-infected astrocytes was disrupted by a significant increase in intracellular calcium when compared to rapamycin or morphine-treated, HIV-infected astrocytes alone. Autophagy inhibition with siATG5 and siATG6 caused a decrease in HIV-p24 production, and significantly increased the secretion of the inflammatory chemokine, RANTES. Morphine co-exposure in siRNA-transfected cells caused a significant decrease in oxidative stress and restored glutamate uptake when compared to morphine-treated, HIV-infected astrocytes. Our data support a role for autophagy on HIV infection in astrocytes and implicate this pathway in maintaining brain homeostasis and immunological control in HIV-1-infected, drug-abusing patients.

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CHANGES IN THE MICROBIOME OF HIV SUBJECTS WITH SUSTAINED VIROLOGICAL CONTROL. Rodríguez-Santiago, R, BS 1, Sánchez, R, BS 1, García-Justiniano, J, BS 1, Pabón-Cruz, E, BS 1, López, P, BS 1, Yamamura, Y, Ph.D. 1, Rivera-Amill, V, Ph.D. 1; 1Ponce Research Institute, Ponce Health Sciences University, Ponce, PR 00716.

The HIV virus and the antiretroviral therapy are capable of inducing prolonged systematic inflammation. This inflammation can cause microbial translocation from the gastrointestinal tract. Along with chronic inflammation, HIV patients suffer a spectrum of comorbidities including mental illnesses. In our study we seek to link the systematic inflammation in HIV+ subjects with the gut, plasma, and saliva microbiomes. We sequenced the 16S to characterize the bacterial composition and predict possible metabolic changes in the microbiomes of HIV infected and healthy patients. We also quantified inflammatory cytokines of each microbiome examined. Our results demonstrate a tendency of lower bacterial richness in the microbiomes of HIV+ subjects. Predicted metabolic routes are also significantly different in HIV+ subjects when compared to uninfected controls. We also observed a difference of inflammatory cytokines in the saliva and plasma of HIV+ positive patients. With these observations we want to correlate microbial translocation/ dysbiosis with HIV infection, comorbid diseases and inflammatory cytokines.

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EFFECTS ON INTEGRINS/CYTOSKELETON BY PARP INHIBITION IN LEUKOCYTES DIMINISH INFLAMMATION AND PROTECTS THE BLOOD BRAIN BARRIER . Rom, S, Ph.D. 1, Zuluaga-Ramirez, V, Ph.D. 1, Reichenbach, NL, BS 1, Dykstra, H, MS 1, Gajghate, S, BS 1, Pacher, P, MD, Ph.D. 2, Persidsky, Y, MD, Ph.D. 1; 1Department of Pathology, Lewis Katz School of Medicine, Temple University, Philadelphia, PA 191402Laboratory of Cardiovascular Physiology and Tissue Injury, National Institutes of Health/NIAAA, Bethesda, MD 20852.

Leukocyte infiltration caused by neuroinflammation into the brain results in blood brain barrier (BBB) dysfunction and contributing to morbidity in traumatic brain injury, stroke, HIV-1 infection and multiple sclerosis. Searching for pathways that diminish the inflammatory potential of leukocytes would avert such injury. In this study, we investigated the notion that selective inhibition of poly(ADP-ribose) type 1 (PARP-1) in leukocytes would reduce their engagement of brain endothelium. Indeed, PARP-1 suppression lessened leukocyte adhesion to and migration across BBB in vitro models and prevented BBB injury. In monocytes, PARP inactivation reduced conformational activation of integrins that plays a key role in their tissue infiltration. Such changes were facilitated by suppression of activation of small Rho GTPases and cytoskeletal rearrangements in monocytes. In vitro observations were verified in vivo showing diminished leukocyte-endothelial interaction after selective PARP suppression in leukocytes and BBB protection. PARP knockout animals exhibited substantial attenuation of inflammatory responses in brain microvasculature and a decrease in BBB permeability. These results suggest PARP inhibition in leukocytes as a novel approach to BBB protection in the setting of endothelial dysfunction caused by inflammation-induced leukocyte engagement.

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MAPPING GENE-NETWORK SIGNATURES OF DIFFERENT NEUROLOGICAL DISORDERS IN AIDS: IMPLICATIONS TO NEUROAIDS . Sagar, Vidya, Ph.D. 1, Martinez, Paola C. 1, Atluri, Venkata Subba Rao , Ph.D. 1, Pilakka-Kanthikeel, Sudheesh , Ph.D. 1, Nair, Madhavan, Ph.D. 1; 1Institute of Neuroimmune Pharmacology, Florida International University , Miami, FL 33199.

The neuroAIDS condition in HIV patients is symptomized by non-specific, multifaceted neurological conditions and pathologies and as such, defining a specific diagnosis/treatment protocols or tools for this neuro-complexity remains elusive. Using an integrated gene network analysis, we discovered that acquired immunodeficiency syndrome (AIDS) shares convergent gene networks with each of 12 neurological disorders selected in this study. Importantly, a common gene network was identified among AIDS, Alzheimer's disease, Parkinson's disease, multiple sclerosis, and age macular degeneration. This unique gene network was compared with a novel, convergent gene network shared by seven major neurological disorders (Alzheimer's disease, Parkinson's disease, Multiple Sclerosis, Age Macular Degeneration, Amyotrophic Lateral Sclerosis, Vascular Dementia, and Restless Leg Syndrome). Both network differed in their gene circuits; however, in large, they involved innate immunity signaling pathways, which suggests commonalities in the immunological basis of different neuropathogenesis. The common gene circuits reported here can elucidate the underlying – and so far unknown – mechanisms of neuropathogenesis during HIV neuro-infection i.e. neuroAIDS. Also it may lead to new paradigm in understanding disease progression, identifying biomarkers, and developing therapies of other neurological disorders.

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PHARMACOLOGICAL ACTIVATION OF AUTOPHAGY PREVENTS LONG-TERM REDUCTION OF ATP LEVELS IN NEURONS EXPOSED TO ANTIRETROVIRALS, METHAMPHETAMINE AND HIV-1 GP120. Sanchez, A.B., Ph.D. 1, Varano, G.P., Ph.D. 1, de Rozieres, C.M. 1, Maung, R. 1, Catalan, I.C. 1, Dowling, C.C. 1, Sejbuk,

N.E 1, Hoefer, M.M., Ph.D. 1, Kaul, M., Ph.D. 1; 1Infectious and Inflammatory Disease Center, Sanford Burnham Prebys Medical Discovery Institute, La Jolla, CA 920372Deaprtment of Psychiatry, University of California San Diego, San Diego, CA 92093.

HIV-1 infection can cause HIV-associated neurocognitive disorders (HAND) despite advanced antiretroviral therapy (ART). In addition, the use of recreational drugs, particularly methamphetamine (METH), seems to aggravate cognitive impairments and may compromise the efficacy of antiretroviral drugs. In the present study, we exposed mixed neuronal-glial cerebrocortical cells to antiretrovirals (ARVs) (zidovudine, nevirapine, saquinavir, and 118-D-24), methamphetamine and HIV-1 gp120 protein for 24 h and 7 days. Subsequently, we assessed neuronal injury by immunocytochemistry, using specific markers for neuronal dendrites and presynaptic terminals. We also analyzed the disturbance of neuronal ATP levels and assessed the involvement of autophagy. ARVs caused alterations of neurites and presynaptic terminals primarily during the 7-day incubation and depending on the specific compounds and their combinations with and without methamphetamine. Similarly, the loss of neuronal ATP was context specific for each of the drugs or combinations thereof, with and without methamphetamine or viral gp120. Loss of ATP was associated with activation of AMP-activated protein kinase (AMPK) and autophagy, which failed to restore normal levels of neuronal ATP. In contrast, boosting autophagy with rapamycin prevented the long-term drop of ATP during exposure to cART in combination with methamphetamine or gp120. Our findings indicate that the overall positive effect of cART on HIV infection is accompanied by detectable neurotoxicity, which in turn may be aggravated by methamphetamine.

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METHAMPHETAMINE-MEDIATED APOPTOSIS IN ASTROCYTES INVOLVES ACTIVATION OF IRE1A, PERK AND ATF6 PATHWAYS OF ENDOPLASMIC RETICULUM (ER) STRESS. SHAH, A, Ph.D. 1, Kumar, A, Ph.D. 1; 1Pharmacology & Toxicology, University of Missouri Kansas City, Kansas City, MO 64108.

Methamphetamine, a psychostimulant drug has been associated with a variety of neurotoxic effects such as induction of pro-inflammatory cytokines/chemokines, oxidative stress and damage to BBB are implicated in MA-mediated neurotoxicity. The ER stress-mediated apoptosis has been implicated in several neurodegenerative diseases. However, its role in MA-mediated neurotoxicity remains largely unknown. The present study was undertaken to assess the detailed mechanisms in the ER stress pathway that is responsible for MA-mediated apoptosis. SVGA astrocytes were used to demonstrate the involvement of IRE1α, ATF6 and PERK pathways in MA-mediated ER stress. MA induced GRP78/BiP and CHOP by 2.7 ± 0.5 fold and 2.3 ± 0.2 fold, respectively at mRNA levels, which was confirmed at protein levels. When assessed in the brains obtained from MA-treated mice, we observed increased levels of BiP and CHOP in addition to various intermediate molecules in the ER stress pathways. The use of siRNA to knockdown intermediate molecules in ER stress pathways demonstrated reduction in CHOP. Finally, involvement of ER stress in MA-mediated apoptosis was demonstrated via MTT assay and TUNEL staining. The apoptosis involved activation of caspase-3 and -8, which was reversed with the use of siRNA against various intermediates. Altogether, the present study is the first report demonstrating mechanistic details responsible for MA-mediated ER stress and its role in apoptosis. It presents ER-stress as a novel therapeutic target in the management of MA-mediated cell death and possibly neuroinflammatory disorders.

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TETHERIN LINKS MICROPARTICLE RELEASE WITH BLOOD-BRAIN BARRIER DYSFUNCTION IN HIV-INFECTED OPIOID USERS. Singh, MV, Ph.D. 1, Singh, VB, Ph.D. 1, Jackson, JW, Ph.D. 1, Kobie, JJ, Ph.D. 1, Bidlack, JM, Ph.D. 1, Schifitto, G, MD 1, Maggirwar, SB, Ph.D. 1; 1Microbiology and Immunology, University of Rochester School of Medicine and Dentistry, Rochester, NY 14642.

Drugs of abuse have been implicated in spread and rapid progression of HIV infection and afflict one-third of all infected individuals in USA. Despite anti-retroviral treatment, chronic immune activation due to residual viral proteins and host inflammatory mediators has lead to increased age-related co-morbidities like neurocognitive impairment, cardiovascular diseases in this population. Here, we are proposing a novel role for host restriction factor Tetherin in regulating pro-inflammatory microparticle (MP) release from activated monocytes which may be implicated in altered blood brain barrier (BBB) function. Our results indicate that HIV-infected, cart treated individuals and HIV uninfected heroin users exhibit reduced levels of Tetherin on monocytes and increased monocyte-derived MPs in plasma. Exposure of healthy cells to HIV-1 protein Tat induced loss of Tetherin expression via proteasome-dependent mechanisms and a concomitant increase in MP release. Importantly, over-expression of degradation-resistant mutant Tetherin in monocytes caused sequestration of fully formed MPs on the cell surface. In addition, mice expressing degradation-resistant Tetherin (NZW/LacJ) show reduced plasma MP levels and a tighter BBB as seen by Sodium Fluorescein assay. Our findings reveal a previously unknown role for tetherin in MP release and are of particular relevance to HIV- and substance abuse-associated neuroinflammatory processes. Future studies will aim at validating these findings using blood samples from HIV infected opioid users and mouse models of HIV infection.

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METHAMPHETAMINE ENHANCES HIV-1 PRODUCTION IN NEURAL STEM CELLS THROUGH NF-KB AND SP-1 SIGNALING PATHWAYS. Skowronska, M, Ph.D. 1, McDonald, M 1, Toborek, M, MD, Ph.D. 1; 1Biochemistry and Molecular Biology Department, University of Miami, Miller School of Medicine, Miami, FL 33136.

HIV infection and METH abuse are often comorbid. Since active METH users were shown to have higher HIV viral load and more severe neurological complications than non-users, synergistic pathological effects were suggested. We hypothesize that one of the mechanisms by which METH enhances HIV-related cognitive decline is METH-potentiated HIV production in neural stem cells (NSCs) that results in impaired neurogenesis. Mouse NSC cell line (NE4C cells), primary mouse progenitor cells, and human stem cells (ReNcell) were infected either with EcoHIV/NL4-3, a chimeric HIV-1 that infects mice or HIV/NL4-3, which is infectious to humans. METH was added simultaneously or 24h before infection. In addition, 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 and HIV production in mouse and human NSCs, respectively. We found that METH induced LTR activation, and that this effect required both NF-κB and SP-1 signaling. In addition, pretreatment with METH decreased differentiation into neuronal lineage and proliferation of EcoHIV infected NSCs. This

study suggests that METH increases HIV production in NSCs through NF-κB/SP-1 dependent activation of HIV-LTR and the subsequent increase in viral genes expression. Such events may underlie METH-induced progression of HIV-associated neurodegeneration and cognitive impairment.

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EXOGENOUS FRACTALKINE TREATMENT MODULATES THE EXPRESSION OF PRO-INFLAMMATORY FACTORS IN THE HIPPOCAMPUS OF ADULT, PRENATALLY STRESSED RATS. Slusarczyk, J, MS 1, Trojan, E, MS 1, Glombik , K, Ph.D. 1, Chamera, K, MS 1, Basta-Kaim, A, Ph.D. 1; 1Department of Experimental Neuroendocrinology, Institute of Pharmacology, Polish Academy of Sciences, Cracow, 31-343.

Recent data show that chemokines play many different functions in the brain, including the control of the neuron-microglia communication and the microglial activation. In this context, fractalkine (CX3CL1) and its receptor (CX3CR1) are the most important because of the specific localization of the ligand - mostly on neurons and its receptor - on microglial cells. Based on our previous observations, which demonstrated disturbances in the CX3CL1-CX3CR1 axis and enhanced brain pro-inflammatory status in adult male rats after prenatal stress procedure (an animal model of depression), the main purpose of the present study was to determine whether the exogenous fractalkine administration may modify the evoked by stress biochemical changes in offspring. Adult 3-month-old rats (control and prenatally stressed) were treated i.p.v. with exogenous fractalkine. 7 days after CX3CL1 administration the animals were decapitated and the biochemical studies were conducted in hippocampus. We measured the expression and protein level of pro-inflammatory cytokines (IL-1β, TNF-α, IL-18, IL-6) and chemokine (CCL2) using qRT-PCR and ELISA methods. The obtained data show that fractalkine treatment reversed prenatal stress-evoked changes in the hippocampal expression and protein levels of pro-inflammatory factors in adult animals. Obtained results suggest that increased inflammatory processes caused by prenatal stress in the brain may be related to the disturbances in fractalkine signaling.

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METHAMPHETAMINE AFFECTS T CELL SIGNATURE IN A CHRONIC LYMPHOCYTIC CHORIOMENINGITIS VIRAL INFECTION MODEL. Sriram, U 1, Cenna, JM 1, Haldar, B 1, Gofman, L 1, Hill, BL 1, Fernandes, NC 1, Potula, R 1; 1Department of Pathology and Laboratory Medicine, Lewis Katz School of Medicine at Temple University, Philadelphia, PA 191402VSH, Verity Software House, Topsham, ME 04086-0247.

Methamphetamine (METH) is a highly addictive psychostimulant that not only affects the brain and cognitive functions but also greatly impacts the host immune system, by increasing susceptibility to infections and exacerbating disease. Evidence is gathering about METH abuse and increased incidence of HIV and other viral infections. Lessons from lymphocytic choriomeningitis virus (LCMV) chronic infection models have been very useful to study HIV pathogenesis. We used the LCMV mouse model in a chronic METH environment and studied the classic T cell signature (activation and inhibitory markers and cytokine profile) induced by infection. We demonstrate that METH significantly increased programmed death-1 (PD-1) expression on T cells, a key molecule known to inhibit T cell function and cause exhaustion during infection. Serum interleukin-2 (IL-2), a classic T cell cytokine, was prominently

decreased in METH-exposed infected mice. In addition, METH altered the serum pro-inflammatory and Th2 cytokine profiles. METH also affected the polyfunctionality of T cells during infection. Correlation patterns of splenic T cell functions such as cytokine production and degranulation were also affected upon METH exposure. METH altered the differentiation of effector/memory T cell subsets during infection. Further, PD-1 expression on T cell effector/memory subsets and correlation with T cell functional markers was affected by METH. Collectively, our data suggests that METH alters systemic and peripheral immune responses and modulates the T cell signature during chronic viral infection.

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COMPARISON OF ANALGESIC ACTION OF NOVEL OPIOID-NK1R BIFUNCTIONAL LIGANDS IN ACUTE AND NEUROPATHIC PAIN IN MICE. Starnowska, J 1, Guillemyn, K 2, Betti, C 2, Makuch, W 1, Ballet, S 2, Mika, J 1, Przewlocka, B 1; 1Department of Pain Pharmacology, Institute of Pharmacology PAS, Krakow, 31-3432Department of Organic Chemistry, Vrije Universiteit Brussel, Brussels, B-1050.

Opioids are commonly used as painkillers in clinical context, yet they do not cover every aspect of efficient pain treatment. Addressing such problems as weak analgesic effect of opioids in neuropathy is of great importance. Neuropathic pain conditions are linked with increased secretion of substance P and elevated expression of its receptor (NK1R). On this basis, targeting endogenous systems via two ways: opioid receptors agonism and NK1R antagonism, was proposed. Herein we tested the analgesic efficacy of 4 bifunctional ligands (‘hybrids’) administered intrathecally in mice in comparison with opioid parent compound. Two of the hybrids are tested for the first time, while the other two were tested by us in rats. We tested hybrids in two models of pain in mice: acute pain (tail-flick test) and neuropathic pain (von Frey and cold plate tests) caused by chronic constriction injury (CCI). The drugs did not influence the motor functions of the mice, as was proved with Rota Rod test. Interestingly, we observed differences in analgesic actions of drugs tested depending on a model: 3 out of 4 bifunctional ligands were not efficient in acute pain being potent analgesics in neuropathic pain, and opioid parent compounds demonstrated opposite tendency. This suggests substantial contribution of overactivated NK1 system to neuropathy symptoms, as well as justifies the direction of searches for novel therapies.

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CLINICAL AND FUNCTIONAL CHARACTERIZATION OF SINGLE NUCLEOTIDE POLYMORPHISMS (SNPS) WITHIN THE HIV-1 LONG TERMINAL REPEAT (LTR) COUP BINDING SITE ASSOCIATE WITH INCREASED VIRUS PERSISTENCE IN THE DREXEL MEDICINE CARES COHORT . Sullivan, N, BS 1, Nonnemacher, MR, Ph.D. 1, Pirrone, V, Ph.D. 1, Feng, Rui, Ph.D. 2, Moldover, B, Ph.D. 3, Dampier, W, Ph.D. 1, Passic, S, MS 1, Williams, J, BS 1, Aiamkitsumrit, B, Ph.D. 1, Zhong, W, BS 1, Blakey, B, MS 1, Shah, S, Ph.D. 1, Jacobson, JM, MD 4, Wigdahl, B, Ph.D. 1; 1Department of Microbiology and Immunology, Institute for Molecular Medicine and Infectious Disease, Drexel University College of Medicine, Philadelphia, PA 191022Department of Biostatistics and Epidemiology, Center for Clinical Epidemiology and Biostatistics, University of Pennsylvania School of Medicine, Philadelphia, PA 191043B-Tech Consulting, LTD, B-Tech

Consulting, LTD, Philadelphia, PA 191304Department of Medicine, Division of Infectious Disease and HIV Medicine, Drexel University College of Medicine, Philadelphia, PA 19102.

The HIV-1 LTR is continuously under selective pressure and LTR SNPs can alter viral transcription in a cell-type dependent manner. To elucidate the clinical and functional impact of HIV-1 SNPs, the Drexel Medicine CNS AIDS Research and Eradication Study (CARES) Cohort conducted a prospective, longitudinal study on >500 HIV-1-infected patients. Numerous SNPs were strongly correlated with clinical disease parameters, such as CD4+ T-cell count and viral load. Of interest, LTR position 108, a COUP/AP1 binding site, increased in frequency in patients with high viral loads and low CD4+ T-cell counts. Electrophoretic mobility shift assays (EMSAs) functionally demonstrated differential transcription factor (TF)/DNA binding profiles with Jurkat and U-937 nuclear extract (NE) when the nucleotide at position 108 is changed. The binding site with an A at position 108 (108A) formed 3 complexes while a G at this position (108G) formed 4 complexes with Jurkat NE. Three complexes were formed with both constructs when U-937 NE were used in the EMSAs. JASPER and supershift EMSAs support the presence of GATA-2, ETS-1, AP-1, and COUP binding to the sequences surrounding 108. Transient expression analyses with an LAI LTR containing a 108G showed increased transcription as compared to 108A in U-937 but not in Jurkats. This observation correlates with the increased viral load and persistence associated with the 108G change in specific individuals. These results demonstrate that mutations are occurring in individuals on antiretroviral therapy that are clinically and functionally important.

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EFFECTS OF SRI-30827 AND SRI-20041 ON THE ALLOSTERIC MODULATION OF HIV-1 TAT BINDING SITES ON HUMAN DOPAMINE TRANSPORTER . Sun, W.L., Ph.D. 1, Quizon, P.M., BS 1, Ananthan, S., Ph.D. 2, Zhang, W., Ph.D. 3, Zhan, C.G., Ph.D. 3, Zhu, J., MD, Ph.D. 1; 1Department of Drug Discovery and Biomedical Sciences/South Carolina College of Pharmacy, University of South Carolina, Columbia, Columbia, SC 292082Department of organic Chemistry, Southern Research Institute, Birmingham, AL 352553Molecular Modeling and Biopharmaceutical Center and Department of Pharmaceutical Sciences/College of Pharmacy, University of Kentucky, Lexington, KY 40536.

Our published work has demonstrated that Tat-induced inhibition of dopamine (DA) transporter (DAT) is mediated by allosteric binding site(s) on DAT, not the interaction with the DA uptake site. Mutations of Tyrosine470 and 88 (Y470H and Y88F) of human DAT (hDAT) have been shown to attenuate Tat-induced inhibition of DAT. This study assessed whether SRI-20041 and SRI-30827, via an allosteric modulation of tyrosine470 and 88 sites of hDAT, pharmacologically block Tat binding to DAT. We performed [3H]DA uptake and [3H]WIN35,428 binding assays in PC12 cells transiently transfected with WT and mutated hDAT in the presence of SRI-20041, SRI-30827, cocaine or Tat. Tat (140 nM) induced a 35% reduction of [3H]DA uptake in WT hDAT but not in Y470H and Y88F. SRI-20041 and SRI-30827 produced a 30% increase in IC50 value for cocaine inhibiting [3H]DA uptake in WT hDAT, however, the effect of the two SRI-compounds on cocaine IC50 was attenuated in Y470H and Y88F. Cocaine-induced dissociation rate in WT was similar to that in Y88F, but was decreased in Y470H. Compared to cocaine alone, the addition of SRI-20041 or SRI-30827 following the addition of cocaine slowed the dissociation rate of [3H]WIN35,428 binding in WT hDAT, however, the effect of SRI

compounds on cocaine-induced dissociation was attenuated in Y470H and Y88F. These results indicate that tyrosine470 and 88 may act as allosteric modulatory sites on DAT responsible for SRI-20041, SRI-30827, and Tat, which may beneficial to developing therapeutic agents for blocking Tat binding on DAT.

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NEURON-ASTROCYTE INTERACTION IN THE PATHOGENESIS OF HIV-1/AIDS-ASSOCIATED NEUROPATHIC PAIN. Tang, SJ, Ph.D. 1, Liu, X, Ph.D. 1, Yuan, S, MD, Ph.D. 1, Shi, Y, Ph.D. 1; 1Department of Neuroscience and Cell Biology, University of Texas Medical Branch, Galveston, TX 77555.

Neuropathic pain is a common neurological complication that severely deteriorates the life quality of HIV-1/AIDS patients, but effective therapies are not available. We are interested in understanding the pathogenic mechanism for the ultimate development of rationale-based therapeutic approaches. Our previous studies reveal that astrocytes are specifically activated in the spinal dorsal horn (SDH) in the 'pain-positive' HIV patients but not in the 'pain-negative' patients, indicating a critical role of reactive astrocytes in the pathogenesis of HIV-associated pain. The current study is to elucidate how the astrocytes are activated during the pathogenesis. Using a mouse model that develops similar pain pathologies to that of 'pain-positive' HIV patients, we show that HIV-1 gp120 induces rapid Wnt5a up-regulation and subsequent astrocyte activation in the SDH. We want to test the hypothesis that in response to gp120 stimulation, Wnt5a is secreted from SDH neurons (the major cell type expressing Wnt5a) to activate astrocytes via its co-receptor ROR2. We have used the conditional knockout approach to delete Wnt5a in neurons or ROR2 in astrocytes. Our results indicate that deleting Wnt5a in neurons or ROR2 in astrocytes not only blocks the expression of go120-induced pain but also astrocyte activation in the SDH. The findings suggest that Wnt5a is a neuron-to-astrocyte signal that is critical for HIV-associated astrocyte activation and pain pathogenesis.

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IFNB PROTECTS NEURONS IN A CCL4-DEPENDENT FASHION AGAINST HIV-1 GP120-INDUCED INJURY. Thaney, V, BS 1, Kaul, M, Ph.D. 1, Hoefer , M, Ph.D. 1; 1Immunity and Pathogenesis Program, Sanford-Burnham Prebys Medical Discovery Institute, La Jolla, CA 92037.

HIV-1 invades the CNS soon after peripheral infection and can result in HIV-associated neurocognitive disorders (HAND). Type I interferons are critical mediators of anti-viral immune response and IFNβ has been implicated in the control of HIV and SIV infection of the brain. However, the potential role of IFNβ as a neuroprotective factor in the context of HIV/gp120-induced neuronal injury remains to be characterized. In this study, we show in both in vitro and in vivo models that treatment with IFNβ completely abrogates neuronal damage caused by HIV gp120. We also show that HIV/gp120tg mice, expressing the viral envelope protein in the brain, mount an IFN response. HIV/gp120tg mice transiently express IFNβ prior to the development of neuropathology and behavioral impairment. These mice manifest several neuropathological features observed in AIDS brains, such as decreased synaptic and dendritic density, increased numbers of activated microglia, and pronounced astrocytosis. Second, we developed a four-week intranasal IFNβ treatment protocol that completely abrogated neuronal damage in gp120tg mice while increasing IFN-induced gene expression. Moreover, In vitro experiments showed

that IFNβ protects cerebrocortical neurons against gp120-induced neurotoxicity in a concentration-dependent manner through the early induction of CCL4, a known HIV-suppressive factor. Taken together, these results identify IFNβ as a neuroprotective agent able to ameliorate gp120-induced neuronal injury.

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ASTROCYTE-TISSUE INHIBITOR OF METALLOPROTEINASES-1 (TIMP-1) IS REGULATED POST-TRANSCRIPTIONALLY IN HIV-ASSOCIATED NEUROCOGNITIVE DISORDERS BY MIR 155 AND 146B. . Thete, M, MS 1, Ghorpade, A, Ph.D. 1; 1Cell Biology, Immunology and Microbiology, University of North Texas Health Science Center, Fort worth, TX 76107.

The societal burden of the human immunodeficiency virus (HIV-1) infections has prevailed over the past several decades. In the thirty years since the discovery of HIV, an estimated 75 million people have become infected and almost half have died. In 2013, 1.8 million new HIV infections, 29.2 million prevalent HIV cases and 1.3 million deaths were reported. Almost 40-70% HIV+ patients experience neurological problems, together called HIV-associated neurocognitive disorders (HAND). Our previous work has shown the neuroprotective role of TIMP-1. Differential long-term regulation of TIMP-1 in neuroinflammation is relevant to HAND neuropathogenesis. IL-1β activated astrocytes elevated the levels of 12 miRNAs whereas 4 miRNA levels were decreased. BLAST analysis identified that, miR 155, miR 29b, miR 518e each have one, whereas miR 146b has four putative binding sites in the TIMP-1 3'UTR. In this reporter construct, TIMP-1 3'UTR is linked to the firefly luciferase gene. Hence any changes will reflect the ability of TIMP-1 3'UTR regulation via miRNAs. Overexpression of miR 146b, miR 155 and miR 29b consistently decreased TIMP-1 3'UTR-dependent firefly luciferase activity, whereas miR 518e, which was downregulated in the array, did not. Changes in firefly luciferase activity were also reflected at the protein (TIMP-1) levels. Thus, we propose that TIMP-1 can be downregulated in long-term neuroinflammation via miRNAs (miR 155, miR 146b, miR 29b) at the post-transcriptional level.

HIV-INFECTION AND SUBSTANCE OF ABUSE: OXIDATIVE STRESS INDUCES DOPAMINERGIC DYSFUNCTION AND NEUROPLASTICITY. Tiwari, S, MS 1, Kaushik, A, Ph.D. 1, Yndart, A, MS 1, Atluri, V, Ph.D. 1, Jayant, RD, Ph.D. 1, Nair, M, Ph.D. 1; 1Center of Personalized Nanomedicine, Institute of NeuroImmune Pharmacology, Department of Immunology , Florida International University, Miami, FL 33199.

Neurotransmitters are being explored for their important role in personalized health care monitoring. The increasing incidences of infectious disease show dysfunctions in neurotransmitter secretion that alters the psychological stress and timely therapeutics. One of such potential neurotransmitter is dopamine which has an important role in signaling inside the brain serving physiological importance. Dysfunction of dopamine neurotransmitter occurs in human immune deficiency (HIV) infected patients, who additionally administer substances of abuse, resulting in exaggerated neurological impairments. Dopaminergic neurotoxicity leads to physiological stress on HIV-infection and is intensified by substance of abuse. Moreover, administration of highly active antiretroviral therapy (HAART) eradicates HIV but also leads to neuronal toxicity, immune impairments, and side effects on secretion of dopamine. In this report, we show that dopamine level (60% reduction) and altered oxidative stress (50% increment) vary

during HIV infection along with chronic consumption of substance of abuse; methamphetamine and morphine, synergistically. Such alterations damage dopamine rich areas in the brain (neuronal damage) to cause dopamine dysfunction leading to neurotoxicity. Our findings suggest that monitoring dopamine functions during HIV progression could be a crucial diagnostic marker and important for therapeutics optimization.

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PRENATAL STRESS IMPAIRS CHEMOKINE CXCL12 AND ITS RECEPTOR (CXCR4) COMMUNICATION IN ADULT OFFSPRING RATS: BENEFICIAL INFLUENCE OF ANTIDEPRESSANTS. Trojan, E, MS 1, Ślusarczyk, J, MS 1, Głombik, K, Ph.D. 1, Chamera, K, MS 1, Basta-Kaim, A, Ph.D. 1; 1Department of Experimental Neuroendocrinology, Institute of Pharmacology Polish Academy of Sciences, Kraków, 31-343.

The chemokine CXCL12 and its receptor CXCR4 appear to be a key system in neuromodulatory processes in the brain. Thus, it is possible that disturbances in the CXCL12 system may be responsible for disturbances observed in depression. The present study was designed to explore the impact of prenatal stress procedure on the behavioral and biochemical changes in the CXCL12 and CXCR4 expression in the adult offspring rats brains. Moreover, the impact of chronic treatment of fluoxetine and tianeptine on the mRNA expression of both proteins were tested. Pregnant rats were subjected to restraint stress. At 3 months of age, after behavioral verification control and prenatally stressed rats were administered with antidepressants: fluoxetine, tianeptine or vehicle. After that the animals' behavior were tested again and rats were sacrificed. The mRNA expression of CXCL12 and CXCR4 in hippocampi and frontal cortices was measured. The obtained data showed that prenatal stress in adult offspring causes depression-like behavior. The evaluation of the mRNA expression demonstrated increase in the CXCL12 and decrease in the CXCR4 levels in hippocampi and frontal cortices in adult rats offspring after prenatal stress procedure. The chronic treatment of both antidepressants normalized the behavioral disturbances and the mRNA expression of all analyzed proteins. Our study showed that prenatal stress leads to persistent behavioral and biochemical disturbances. Therapeutic efficacy of antidepressants can be related at least in part to the improvement of chemokine-receptor communication.

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EFFECT OF TOBACCO SMOKE AND HIV INFECTION ON MUCOCILIARY CLEARANCE. Unwalla, Hoshang, Ph.D. 1, Chinnapaiyan, S, Ph.D. 1, Periera, T, BS 1, Agudelo, M, Ph.D. 1, Morris, A, MD 2; 1Herbert Wertheim College of medicine, Department of Immunology, Florida International University, Miami, FL 331992Division of Pulmonary, Allergy and Critical care, University of Pittsburgh Medical Center, Pittsburgh, PA 15213.

With the introduction of combination anti-retroviral therapy (cART), HIV has become a treatable but chronic disease. HIV infected patients live the lifespan equivalent to that of normal people. However, despite this progress, some of the comorbidities among HIV-1 infected individuals continue to remain highly prevalent. Among Lung-related comorbidities pneumonia and chronic obstructive pulmonary disease (COPD) are most prevalent when compared to non–HIV-infected adults. This could be due to dysfunctional mucociliary clearance (MCC) that impacts microbial and/or pollutant clearance from the

airways leading to microbial colonization and chronic inflammation. Mucociliary clearance is a primary innate defense mechanism of the airways and protects the host from airborne pathogens, pollutants and allergens. CFTR plays a pivotal role in MCC. TGF-β signaling is upregulated in smokers and in chronic diseases. Our data demonstrate that HIV Tat and cigarette smoke suppress CFTR mRNA and function via a common pathway involving TGF-β signaling. We also demonstrate that HIV can infect primary human bronchial epithelium. This can increase the Tat burden in the airway and directly affect cells involved in MCC. Our data also demonstrates that HIV infection and cigarette smoke, individually and additively suppress CFTR mRNA in NHBE. This is significant since about 60% of HIV infected patients also smoke tobacco. The implications of these findings will be discussed.

NANOCOMPOSITE HYDROGELS FOR NEURO DRUG DELIVERY . Vashist, Arti, Ph.D. 1, Ghosal, Anujit, Ph.D. 2, Gupta, Y.K., MD 3, Ahmad, Sharif, Ph.D. 2, Nair, Madhavan, Ph.D. 1; 1Center for Personalized Nanomedicine,Institute of Neuro immune Pharmacology, Department of Immunology , Herbert Wertheim College of Medicine, Florida International University, MIAMI, FL 331992Materials Research Laboratory, Department of Chemistry, Jamia Millia Islamia, NEW DELHI, 1100253Department of Pharmacology, All India Institute of Medical Sciences, NEW DELHI, 110029.

Nanocomposite hydrogels (NCHs) have been extensively used as drug delivery systems due to their versatile nature and diverse properties to deliver both small molecule and various classes of bio macromolecules. In the present work an antiepileptic drug “sodium phenytoin” was used as a model drug, which is known to precipitate in acidic pH. The objective of the study was to develop pH responsive hydrogel systems, which do not release the drug in acidic medium and bypass the stomach pH, preventing the drug precipitation and release the drug in the physiological pH of the temporal lobe of the brain (pH 7.2). For this purpose, NCHs were prepared by free radical polymerization reaction using Cloisite 30B as a nanofiller. The formation of cross linked networks and hydrogen bonds in the synthesized NCHs were confirmed through FT-IR and SEM analysis. High thermal stability of the NCHs with intercalated structures having an interlayer distance of 1-1.5 nm was determined by TGA, DSC and TEM studies. The UV-Vis spectroscopic studies revealed a higher drug encapsulation ability of NCHs NC-2 (40.9 %) as compared to CGP2-3 (35.1%). Drug release kinetic studies at pH 7.2 indicated a controlled release of phenytoin with zero order release kinetics along with a higher drug retention ability of NCHs. It was proposed that the hydrophobic modification using linseed oil based polyol will provide compatibility to traverse the hydrophobic microenvironment across the blood brain barrier. Based on our preliminary findings we will explore nanogels as CNS drug nanocarrier to eradicate neuroAIDS.

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GAP JUNCTIONS AND HEMICHANNELS ARE KEY REGULATORS OF ANTIRETROVIRAL METABOLISM AND TOXICITY: A NEW ROLE IN NEUROAIDS. Veilleux, Courtney 1, Eugenin, EA, Ph.D. 1; 1Department of Microbiology, Biochemistry, and Molecular Biology, Rutgers University, Newark, NJ 07103.

Although the advent of combination HIV antiretroviral therapy (cART) greatly reduced the incidence of HIV-associated dementia (HAD), HIV-associated neurological disorders (HAND) has increased. Several groups have proposed that the molecular mechanism of HAND involves increased secretion of

neurotoxic and inflammatory factors between brain cells and reduced antiretroviral therapy (ART) penetration in the brain. Our laboratory previously demonstrated that astrocytes are nonproductively infected by HIV, release neurotoxic mediators to neighboring cells, and cause cell death in the brain. These neurotoxic signaling cascades are triggered by HIV infected astrocytes and are facilitated by the opening of gap junctions and hemichannels. Blockade of these channels in astrocytes not only reduces overall toxicity, but greatly diminishes cell death. Therefore, gap junctions and hemichannels blockers present a unique potential therapy for HAND. Importantly, the mechanism by which gap junctions and hemichannels contribute to the intracellular retention and secretion of ART is largely unknown. We hypothesize that gap junctions and hemichannels, in addition to amplifying apoptosis and inflammation, also play a critical role in regulation of ART in the brain. Our results illustrate that blocking of gap junctions or hemichannels affects retention, uptake, and secretion of antiretrovirals in uninfected and HIV infected astrocytes. These results demonstrate a potential role of gap junctions and hemichannels in ART regulation. This research further demonstrates the potential therapeutic advanta

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SIGMA-1 RECEPTOR/COCAINE INTERPLAY IN CATHEPSIN B SECRETION BY HIV INFECTED MACROPHAGES

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Monocyte derived macrophages (MDMs) serve as viral reservoirs and promote inflammation in the brain exacerbating HIV-associated neurocognitive disorders (HAND). One MDM factor involved in neuronal apoptosis is cathepsin B, a lysosomal cysteine protease activated by HIV infection. Cocaine potentiates cathepsin B secretion and neurotoxicity from HIV-infected MDMs by an unknown mechanism. It is possible that activation of sigma-1 receptor (Sig-1R), which increases HIV replication, inflammation, and neuronal death, might be involved. We hypothesize that cocaine heightens HIV-induced cathepsin B secretion by MDMs through Sig-1R activation. MDMs isolated from seronegative donors (n=3) were infected with HIV-1ADA and exposed to 10 µM cocaine for 12 days. Sig-1R was measured from MDMs lysates while cathepsin B and HIV-p24 antigen were measured from supernatants by ELISA. For in vivo studies, HIV-infected MDMs were injected to mice striatum (HIVE) (n=12) followed by intraperitoneal injection of 15mg/Kg cocaine for 14 days. Sig-1R and cathepsin B were quantified by Western blot. In vitro results showed an increase in cathepsin B secretion in the infected cocaine treated group ($p \leq 0.001$) compared to controls. Sig-1R expression did not change. Infected striatum from HIVE mice treated with cocaine show increased cathepsin B, Sig-1R expression ($p=0.04$) as compared to infected or uninfected controls. Results were confirmed in post-mortem brain tissue from patients. Our findings provide evidence that cocaine/Sig-1R and cathepsin B are correlated and pose for potential therapeutics

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LAB-ON-A-CHIP TOOL FOR MODELING BIOLOGICAL BARRIERS. Walter, FR, Ph.D. 1, Valkai, S, Ph.D. 1, Kincses, A, MS 1, Veszelka, S, Ph.D. 1, Ormos, P, Ph.D. 1, Deli, MA, MD, Ph.D. 1, Dér, A, Ph.D. 1; 1Biological Research Centre, Hungarian Academy of Sciences, Institute of Biophysics, Szeged, 6726.

Models of biological barriers are important to investigate physiological functions, transport mechanisms, pathologies and drug delivery. The aim of this study was to design and manufacture a simple but versatile lab-on-a-chip device, which allows a complex investigation of blood-brain barrier functions. A PDMS based biochip with integrated gold electrodes and with a possibility to connect to a peristaltic pump, was built. The structure of the device allowed a constant visual observation of cell growth. The chip was applied to monitor and characterize two cell culture based models of the blood-brain barrier: hCMEC/D3 human brain endothelial cell line and primary rat brain endothelial cells co-cultured with primary rat astrocytes and rat brain pericytes. The following functions and measurements were enabled, and many of them simultaneously: flow of culture medium mimicking shear stress of the blood flow on endothelial cells; visualization of cells by microscopy; real-time transcellular electrical resistance monitoring; permeability measurements for fluorescent marker molecules and immunohistochemistry. The triple primary co-culture blood-brain barrier model was assembled on a lab-on-a-chip device and investigated under fluid flow for the first time. The integrated lab-on-a-chip measuring chamber proved to be suitable for resistance measurement, permeability tests and cell visualization for all the models tested. Such a versatile tool is expected to facilitate the kinetic investigation of various biological barriers.

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INHIBITION OF THE HIV RESTRICTION FACTORS CONTRIBUTES TO MORPHINE WITHDRAWAL-MEDIATED ENHANCEMENT OF HIV REPLICATION IN MACROPHAGES . Wang, X 1, Li, J-L, Ph.D. 1, Ma, T-C 1, Petovic, J.L. 1, Peng, J-S, Ph.D. 2, Zhou, W, MD, Ph.D. 2, Ho, W-Z, MD, M.Ph. 1; 1Department of Pathology and Laboratory Medicine, Temple University Lewis Katz School of Medicine,, Philadelphia, PA 191402Division of Virology, Wuhan Centers for Disease Prevention & Control, Wuhan, 430015.

Opioid withdrawal is a crucial and recurring event during the course of opioid abuse, which has a negative impact on the immune system. We previously demonstrated that both abrupt withdrawal (AW) and precipitated withdrawal (PW, blocking opioid receptors by treatment with naloxone after morphine cessation) facilitates HIV infection of human T lymphocytes. In this study, we examined the impact of AW or PW on HIV infection of human blood monocytes-derived macrophages. We observed that AW and PW enhanced the susceptibility of macrophages to HIV infection. In addition, both AW and PW induced HIV replication in the latently infected myeloid cells (U1 and OM10.1). Investigation of mechanisms responsible for these observations showed that AW and PW could activate HIV-LTR promoter as evidenced by increased LTR-driven CAT activity. Further, the enhancing effect of AW and PW on the virus was associated with the inhibition of the HIV restriction factors (anti-HIV microRNAs: miR-28, miR-125b, miR-150, miR-17 and miR-20a; MIP-beta; and APOBE3G) in macrophages. These findings indicate that opioids use impairs intracellular innate anti-HIV mechanism(s) in macrophages, contributing to cell susceptibility to HIV infection.

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BRAIN-SPECIFIC OVEREXPRESSION OF GLUTAMINASE C INDUCES NEUROINFLAMMATION, SYNAPTIC DAMAGE AND DEMENTIA IN MICE. Wang, Y 1, Li, Y 1, Huang, Y 1, Zheng, J 1; 1University of Nebraska Medical Center, College of Medicine, Omaha, NE 68198.

Glutaminase is the enzyme that converts glutamine into glutamate. Glutamate is essential for proper brain functioning but is toxic at excess levels and has a key role in the pathogenesis of neurodegenerative diseases, including HIV-1 associated neurocognitive disorders (HAND). The detailed mechanism of glutamate-mediated neurotoxicity remains unclear in vivo. Our previous data revealed upregulation of glutaminase C (GAC) in the postmortem brain tissues of patients with HAND. Therefore, we hypothesize that GAC dysregulation in brain is sufficient to induce brain inflammation and dementia relevant to HAND. Using a brain-specific GAC overexpression mouse model, we found the marker for brain inflammation, the glial fibrillary acidic protein, was increased in the brains of GAC-overexpression mice; while the synapse marker, synaptophysin, was decreased. This suggests prolonged neuroinflamamtion and synaptic damage. To study the functional impact of GAC overexpression, we performed Morris Water Maze (MWM) test and Contextual Fear Conditioning test to determine the learning and memory of mice. GAC-overexpression mice performed poorer than control mice in both tests, indicating that overexpressing GAC in mouse brain impaired the learning and memory. Hippocampal long-term potentiation of GAC-overexpression mice was diminished. Memantine treatment partially reversed the performance of GAC-overexpression mice in MWM test. Together, these data suggest that dysregulated GAC in mouse brain causes prolonged inflammation, synaptic damage and dementia.

RECEPTOR-INDEPENDENT MECHANISM OF HIV GP120 NEUROTOXICITY. Wenzel, ED, BS 1, Taraballi, F, Ph.D. 2, Caragher, SP 1, Avdoshina, V, MD, Ph.D. 1, Bachis, A, Ph.D. 1, Mocchetti, I, Ph.D. 1; 1Laboratory of Preclinical Neurobiology, Georgetown University, Washington, DC 200572Nanomedicine, Houston Methodist Research Institute, Houston, TX 77030.

Synapto-dendritic simplification is seen in a subset of Human Immunodeficiency Virus 1 (HIV) positive individuals. Considerable experimental evidence indicates that HIV protein, gp120, is directly responsible for this neurotoxicity through an interaction with chemokine receptors, CCR5 or CXCR4. Yet, the molecular mechanisms leading to these pathological features are still unknown. Gp120 internalization and accumulation is an important potential mechanism of neurotoxicty. We used conditional dynamin 1/2/3 knockout (KO) fibroblasts to confirm receptor- and dynamin-dependent endocytosis of gp120. Abolished endocytosis in KO cells supported gp120 receptor-dependent internalization. To determine whether internalization/accumulation of gp120 is neurotoxic, we utilized gp120-loaded mesoporous silica nanoparticles (gp120MSN). Primary rat cortical neurons were treated with gp120 or gp120MSN alone or in combination with a receptor antagonist. Gp120MSN failed to activate Erk signaling, suggesting that the nanoparticles bypass the chemokine receptors. After 24 hrs, toxicity was evaluated by quantification of propidium iodide positive neurons and measurement of neurite length. Both gp120 and gp120MSN caused profound neurite pruning and cell loss. However, only gp120-mediated, but not gp120MSN-mediated, toxicity was blocked by the chemokine antagonist. Our data suggest that gp120 endocytosis is the initial step allowing for neurotoxicity, but is not the toxic event itself and that intracellular accumulation is crucial for its neurotoxic effect.

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DIFFERENTIAL NEUROTOXICITY INDUCED BY HIV PROTEASE INHIBITORS IN VITRO. Williams, K, Ph.D. 1, Li, J 1, Lee, R 1, Chuang, E 2, Jordan-Sciutto, K, Ph.D. 1, Espinoza, C, Ph.D. 1; 1School of Dental Medicine , University of Pennsylvania, Philadelphia, PA 191042School of Medicine , University of Pennsylvania , Philadelphia, PA 19104.

In the post antiretroviral therapy (ART) era, 30-50% of HIV patients present with HIV associated neurocognitive disorders (HAND). Previous work in our laboratory demonstrated that two first generation HIV proteases inhibitors (PI), ritonavir and saquinavir, led to synaptic damage and neuronal death in vitro. Intriguingly, previous studies have shown certain antiretroviral drugs induced endoplasmic reticulum (ER) stress in peripheral cell types, resulting in chronic dysregulation of the unfolded protein response (UPR). Importantly, UPR was shown to alter the expression and activity the β-site APP cleaving enzyme-1 (BACE1), leading to altered amyloid precursor protein (APP) processing. In an effort to understand potential contribution of ART drugs to continued HAND prevalence, we herein assessed the impact of two newer PIs, darunavir and lopinavir, on UPR activation and neurotoxicity. Primary rat neuroglia cultures at 14 days in vitro were treated with increasing doses of darunavir or lopinavir for 4, 8, or 24 hours. Lopinavir treatment increased BiP and BACE1 at 4hrs but not later time periods. Lopinavir treatment also led to decreases in mitochondrial membrane potential after 2 hours of treatment. Finally, darunavir did not appear to induce neurotoxicity or UPR activation. Taken together, our data suggested that lopinavir, but not darunavir, induced UPR activation and neurotoxicity and raise the possibility that specific PIs may be contributors to damage. Thus, our studies support examination of members of the PI class of ART for their neurotoxic profiles is warranted.

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TESTIS-SPECIFIC LACTATE DEHYDROGENASE (LDH-C4) IN THE BRAIN MAY CONTRIBUTE TO PREVENT CNS AGING. Yang, Wang, MD, Ph.D. 1, Lian, Wei, MS 1, Linna, Wei, Ph.D. 1, Xiao, Li, Ph.D. 1, Lina, Xu, Ph.D. 1, Dengbang, Wei, Ph.D. 1; 1Research Center for High Altitude Medicine, Qinghai University, Xining, China, 810016.

The human brain is an organ with high energy requirements, which mainly relies on aerobic metabolism. It has been studied that the increased brain lactate levels as a marker in CNS aging were caused by a shift in transcriptional activities of the lactate dehydrogenases (LDH) to promote pyruvate to lactate conversion. Separation of the five tetrameric LDH isoenzymes revealed an increase of those dominated by the Ldh-a product and a decrease of those rich in the Ldh-b product, which, in turn, increases pyruvate to lactate conversion. Under conditions of increased lactate production, the use of lactate in the blood as an energy source in the brain increases at the expense of blood glucose. It is thought that an activity-regulated lactate shuttle from astrocytes to neurons would allow neurons to benefit from lactate. LDH-C4 in mammals was previously thought to be expressed only in testis and spermatozoa. Surprisingly, we identified that Ldh-c also expresses in the brain of plateau pika. LDH-C4 expressed in the brain may play the crucial role in anaerobic glycolysis and generate ATP since this is the role of LDH-A4 in most species. Compared with LDH-A4 and LDH-B4, LDH-C4 had a low Km for pyruvate;

and the affinity of LDH-C4 for pyruvate is 90-fold higher than that for lactate. The properties of pika LDH-C4 was beneficial to catalyze the conversion pyruvate to lactate even in the high concentration of lactate. These could bring an inspiration that the exogenous supplement of LDH-C4 as a therapeutic medicine may prevent aging or treat neurodegenerative disease caused by aging.

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THC-MEDIATED ALTERATIONS IN GLOBAL GENE EXPRESSION IN LYMPHOCYTES. Yang, X, Ph.D. 1, Bam, M, Ph.D. 1, Nagarkatti, PS, Ph.D. 1, Nagarkatti, M, Ph.D. 1; 1School of Medicine, University of South Carolina, Columbia, SC 292092Research, Dorn VA Medical Center, Columbia, SC 29209.

Several studies have suggested that marijuana may de-regulate the immune response. In order to exam the effect of marijuana on global gene expression in lymphocytes, we used RNA-Seq to identify differentially expressed transcripts. In this study, mice were pretreated with THC, the main bioactive component in marijuana, and then challenged by staphylococcal enterotoxin B (SEB). RNA abundance in total popliteal lymphocytes as well as CD4+ T cells was quantified by RNA-seq. We found that the expression of many transcripts was altered by THC in both total lymphocytes and CD4+ T cell subpopulation. A unique finding of this study was that the expressions of many miRNA precursors, including miR-17/92 cluster, miR-374/421 cluster, miR-210 and miR-146a, were dramatically affected by THC. However, the majority of these miRNAs are newly identified with unknown functions. These novel miRNAs may have important roles in immune response. Interestingly, we found that the expression of some miRNAs and their potential target genes were oppositely expressed in THC treated cells, suggesting that miRNA expression might play an important role in THC-mediated immune regulation. In addition, THC treatment also caused alternative promoter usage and splicing. The functions of those altered transcripts are mainly related to immune response and cell proliferation. This study shows that THC has pleiotropic effects in the immune system through various mechanisms.

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SMALL MOLECULE ONC201/TIC10 INHIBITS HIV-1 REPLICATION AND INTEGRATION. Zhao, R, BS 1, Wu, B, MD 1, Huang, Y, MD, Ph.D. 1, Zheng, JC, MD 1; 1Pharmacology and Experimental Neuroscience, University of Nebraska Medical Center, Omaha, NE 68105.

HIV-1 continues to form reservoirs in the lymphoids, gut and central nervous system, representing a significant challenge for viral eradication. FOXO3a, a transcription factor critical for immune homeostasis, is known to inhibit latent HIV-1 reservoirs. Recent drug development has provided TIC10 as the first potent and stable small molecule FOXO3a activator capable of crossing blood brain barrier. We hypothesize that targeting FOXO3a through TIC10 will inhibit HIV-1 in its reservoir cell types. We used human primary microglia, monocyte-derived macrophages and peripheral lymphocytes infected with HIV-1 ADA or IIIB. Viral infection and integration were monitored by HIV-1 reverse transcriptase activity, p24, and two step Alu-based nested PCR, respectively. As expected, TIC10, but not its inactive isomer, potently inhibited HIV-1 replication and reduced integrated DNA in infected cells in a dose-

dependent manner, suggesting that the antiviral activity is specific to TIC10. Interestingly, unlike tumor cell lines, which were sensitive to TIC10-induced cell death, primary macrophages were resistant to TIC10-induced cell death. The reduced levels of HIV-1 replication and integration in infected cells after TIC10 treatment were associated with FOXO3a activation, TNF-related apoptosis inducing ligand expression and cleavages of caspase 3, indicating that TIC10 inhibits HIV-1 replication through modulation of FOXO3a and the related apoptotic signaling. Together, these data suggest that TIC10 can be a promising drug candidate to combat latent HIV-1 infection in reservoir cell types.

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THE ROLE OF LYMPHOTACTIN AND ITS RECEPTOR IN MICE DIABETIC NEUROPATHIC PAIN MODEL

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Diabetes is responsible for 60% of all deaths worldwide and its most common complication is neuropathic pain. Participation of different cytokines in diabetic neuropathic pain pathology is established, however role of lymphotactin (XCL1) and its receptor (XCR1) remind unclear. The goal was to determinate the changes in the levels of XCL1 and XCR1 during diabetic neuropathy. We also verified the influence of intrathecal XCL1 and XCL1 neutralizing antibody administration on nociceptive transmission. The mice model of diabetic neuropathy was obtained by single intraperitoneal streptozotocin (STZ; 200 mg/kg) injection. The neuropathic pain syndrome was evaluated with von Frey and cold plate tests. Western blot technique was preformed to analysis changes in XCL1 and XCR1 protein levels. XCL1 and its neutralizing antibody were intrathecally injected, and neuropathic pain syndrome was evaluated. STZ administration induced development of allodynia and hyperalgesia parallel with up-regulations of the XCL1 and XCR1 levels. Intrathecal XCL1 administration into naïve mice induced neuropathic pain syndrome, and injections of neutralizing XCL1 antibody into the diabetic neuropathy diminished pain. Additionally, primary microglial culture indicated that microglia are responsible for XCL1 release and XCR1 expression. This study provides evidence for the important role of microglia in the expression of XCL1/XCR1 in diabetes.

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