

each stream ecosystem may be impacted by land use and intensified human expansion. According to the fall macrobenthic results, nearly all sites saw an increase in %EPT, except for Massaponax 208 and Ni Route 1. Even though both Massaponax Creek and the Ni River are under EPA legal limits for phosphate and nitrate concentrations, the phosphate levels usually rose in summer on the Massaponax and winter for the Ni, while nitrates peak during the winter and spring for both streams. TDS increased during the summer and then declined, as precipitation rates fell. Massaponax Creek has higher TDS than the Ni, but that is reversed for TSS. Fecal coliform levels greatly decreased from summer into winter and fall, with both streams remaining under legal limits. This speaks to the recovery of the streams, resulting from repaired sewer lines and decreased erosion risks in surrounding areas specifically near Massaponax Creek. Due to the majority increase in %EPT, decreased concentration of nitrates and phosphates, and reduced fecal coliform levels, the Ni River and Massaponax Creek have generally seen an improvement in overall health.

Medical Sciences

THE ACTIVATION STATUS OF RHEB IN PEMETREXED TREATED HUMAN CANCER CELLS. C. M. Bell, S. Agarwal & R. G. Moran, Dept. of Pharmacology & Toxicology, Virginia Comm. Univ., Richmond, VA 23298. Rheb (Ras homolog enriched in brain) is a small GTPase essential for activating mTORC1 (mammalian target of rapamycin complex 1) when loaded with GTP. mTORC1 activation increases cell growth by promoting protein synthesis and lipogenesis. Tuberous sclerosis protein 2 (TSC2) negatively regulates mTORC1 by stimulating the GTPase activity of Rheb. Pemetrexed (PTX) is a multi-targeted antifolate drug that suppresses tumor growth by obstructing folate metabolism and indirectly activating AMP activated kinase (AMPK). AMPK-activators inhibit cell growth by activation of TSC2 and deactivation of the mTORC1-partner protein, Raptor. We have shown that PTX treatment does not increase the activating phosphorylation of TSC2, but that mTORC1 activity is still significantly decreased. Further, we have also shown that in cells null for p53, more Rheb is associated with Raptor and mTORC1 activity is increased. Thus, two methods have been developed to measure the levels of Rheb-bound nucleotide. Coupled enzymatic assays using nucleoside diphosphate kinase were employed to convert GTP to ATP, and the ATP quantified in a luciferase assay. Corresponding GDP was converted to $\gamma^{32}\text{P}$ -GTP and quantified by reverse phase-HPLC and liquid scintillation counting. Alternatively, cells were metabolically labeled with ^{32}P orthophosphate and the Rheb-bound nucleotides resolved by TLC. These two methods show the levels of Rheb-GTP to be increased in p53 null cells and surprisingly slightly increased in AICAR or PTX treated cells. Further studies aim to elucidate these data, specifically by assaying subcellular compartments of the cell for Rheb activation.

TOWARDS UNDERSTANDING MOLECULAR INTERACTIONS OF ETHYLENEDIOXY COUNTERPARTS OF MDMA AND METHYLONE AT hDAT. F. T. Sakloth¹, F. Del Bello¹, R. Kolanos¹, J. Partilla², P. D. Mosier¹, M. H. Baumann² & R. A. Glennon¹, ¹Department of Medicinal Chemistry, VCU, Richmond, VA 23298 and ²National Institutes of Health, NIDA, Baltimore, MD 21224. MDMA (“Ecstasy”)

and its β -keto analog, methylone (MDMC), are controlled (Schedule I) substances that are popular recreational drugs. They act as releasing agents at dopamine and/or serotonin transporters (DAT and/or SERT). The ethylenedioxy counterparts of MDMA (EDMA) and MDMC (EDMC) are found on the "street" and could represent a potential abuse problem, but there is little information regarding their actions. The goals of this project are to 1) examine these ethylenedioxy analogs at DAT using in vitro (synaptosomal) release assays, and 2) construct homology models of hDAT using the crystal structure of *D. melanogaster* followed by docking studies to determine if differences in potencies might be explained by interactions at DAT. Release data showed that (?)EDMA and (?)EDMC are 8- and 4-fold less potent, respectively, than MDMA (IC_{50} =75 nM) and MDMC (IC_{50} = 133 nM) and that *S*-EDMA (IC_{50} =276 nM) is 50-fold more potent than *R*-EDMA. Modeling studies suggest that potentially unfavorable interactions of the ethylenedioxy group of EDMA (especially its *R*-isomer) with Ser149 might explain its lower potency at DAT. Additionally, the difference in the potency of MDMA and EDMA, and of *S*-MDMA and *S*-EDMA over their lower-potency *R*-isomers seems to be explained by the energy penalty for the binding of the ligands at DAT. EDMA and EDMC are unlikely to be as much of an abuse problem as their methylenedioxy counterparts. [Supported by NIH grant DA 033930.]

ALTERATIONS TO THE SYNAPTIC TRANSCRIPTOME IN RESPONSE TO ETHANOL-INDUCED SENSITIZATION. Megan A. O'Brien & Michael F. Miles, Department of Pharmacology & Toxicology, Virginia Commonwealth University, Richmond VA 23298. It is well established that mRNA can be transported to neuronal distal processes, where it can undergo localized translation regulated in a spatially restricted manner in response to stimulation, potentially playing a role in synaptic plasticity that results in long-term adaptive brain responses. In order to investigate our hypothesis that ethanol behavioral sensitization results, at least in part, from alterations in the trafficking of mRNAs to distal processes, we profiled the synaptic transcriptome utilizing two distinct methodologies: mRNA microarray and RNA-Seq. By using two independent techniques, which employ different molecular principles, we were able to validate data processing schemes as well as differential expression results. We found that there was a significant overlap between these analyses (p-value < 1.0E-16), where 70% of the genes determined by RNA-Seq to be differentially expressed between the synaptic and cytosolic fractions were also deemed significant by microarray analysis. Expression data was then analyzed to identify 229 synaptically targeted candidate genes differentially expressed as a result of ethanol treatment. Examination of gene expression patterns revealed a number of genes whose levels were altered in response to acute ethanol, but habituated in response to repeated, sensitizing ethanol treatment. Functional over-representation analysis revealed enrichment for genes associated with the endoplasmic reticulum and the extracellular matrix. Support provided by NIAAA grants U01AA016667, P20AA017828, and F31AA021035.

INHIBITION OF MONOACYLGLYCEROL LIPASE: THERAPEUTIC POTENTIAL WITH LIMITED CANNABIMEMETIC EFFECTS. B. Ignatowska-Jankowska¹, M. Mustafa¹, S. Ghosh¹, M. Niphakis², R. Abdullah¹, J. Wilkerson¹, J. Wiley³, B. Cravatt², & A. Lichtman¹, ¹ Dept. of Pharmacology & Toxicology, VCU, ² The Skaggs Institute for Chemical Biology & Dept. Of Chemical Physiology, TSRI, ³Research Triangle

Institute, RTP. The endocannabinoid 2-arachidonoyl glycerol (2-AG) plays a role in many physiological processes, and it is degraded by monoacylglycerol lipase (MAGL). While increase in 2-AG signaling by preventing its degradation is a promising strategy in treatment of pain, and several other disorders, its function and effects *in vivo* remain unclear. MJN110 is a novel, selective inhibitor of MAGL that allows for examination of increase in 2-AG, without affecting other endocannabinoid levels. In the present study we tested MJN110 in a murine model of inflammatory pain induced by carrageenan as well as tetrad and drug discrimination assays. MJN110 showed high efficacy and potency to produce anti-allodynic and anti-edematous effects in the pain model [ED₅₀ (95% confidence intervals, C.I.)=0.26 (0.14-0.47) mg/kg]. MJN110 at high doses produced antinociception and increased locomotor activity, but did not induce catalepsy or hypothermia. In drug discrimination assay, MJN110 fully substituted for CP55,940, [ED₅₀ (95% C.I.)=0.84 (0.69-1.0) mg/kg], indicating that it produced cannabimimetic interoceptive effects. The antinociceptive effects of MJN110 in the carrageenan assay were 2.4 (1.6-4.4) [potency ratio (95% C.I.)] more potent than in the drug discrimination. MJN110 significantly elevated 2-AG but not other endocannabinoid levels in the brain. Findings are in agreement with *in vitro* research showing that MJN110 is highly potent and selective MAGL inhibitor *in vivo*. The results indicate that MAGL inhibition with MJN110 reverses inflammatory pain at lower doses than those that produce cannabimimetic side effects.

THE ABILITY TO MODULATE AND INFLUENCE IMMUNE CELLS TO IMPACT TUMORS IN DIVERSE CONTEXTS. Se W. Jeong & Timothy N. J. Bullock, Department of Pathology, University of Virginia, Charlottesville, VA 22908. Metastatic melanoma has one of the poorest prognoses for patients as the median survival ranges from six to nine months. Nonetheless, there has been significant progress made over the past several years in which immunotherapy have contributed considerably. Celldex Therapeutics has developed a fully human monoclonal antibody which targets CD27 (CDX-1127), a potent costimulatory molecule that can drive lymphocyte proliferation and expansion. CDX-1127 is able to mimic CD70, which serves as the ligand for CD27. The interaction thus creates an appropriate T cell receptor signal to drive T cell activation. There is a significant interest in using CD27 as a therapeutic model as data have shown strong evidence of anti-tumor response and immunity in recent studies. Whereas Celldex administers clinical trials, our immunology laboratory receives patient samples throughout the treatment for flow cytometry analysis of key immunological molecules, identifying B and T cells and to enumerate specific subsets of T cells. One of the more interesting results from CDX-1127 phase I clinical trial is that the MHC class II surface receptor HLA-DR expression increases in majority of the patients, which suggests the immune system is becoming more active upon treatment. In addition, regulatory T cells significantly decreased after the CDX-1127 treatment further implicating the potential for CD27 target therapy for melanoma patients

DIACYLGLYCEROL LIPASE BETA: NEW EVIDENCE FOR INFLAMMATORY AND NEUROPATHIC PAIN RELIEF IN MICE. J. Wilkerson¹, S. Ghosh¹, K. Hsu², B. Cravatt² & A. Lichtman¹,¹Dept. of Pharmacology & Toxicology, Virginia Commonwealth University, Richmond, VA 23298, ²The Skaggs Institute & TSRI, La

Jolla, CA 92037. Diacylglycerol lipase (DAGL), the enzyme responsible for generation of 2-arachidonoylglycerol, represents a component of the endogenous cannabinoid system as a potential therapeutic target to treat pain. As DAGL- β inhibition leads to decreases in lipopolysaccharide (LPS)-induced prostaglandins & proinflammatory cytokines, we hypothesized that inhibition of this enzyme will reverse nociceptive behavior in a mouse model of inflammatory pain. We tested DAGL- $\beta^{(-/-)}$ & wild type (WT) mice treated with the selective DAGL- β inhibitor KT109 in the inflammatory model of intraplantar LPS (2.5 μ g) pain. LPS produced robust increases in sensitivity to light mechanical touch, or allodynia, & increased thermal sensitivity, or thermal hyperalgesia in WT mice. Systemic & local intraplantar injection of KT109 reversed LPS-induced allodynia in a time- & dose-dependent manner. DAGL- $\beta^{(-/-)}$ mice displayed reductions in LPS-induced allodynia. KT109 administration into the contralateral paw did not reduce LPS-induced hyperalgesia or allodynia, suggesting that these effects occurred locally. Neither intrathecal nor intracerebroventricular injection of KT109 reversed LPS-induced allodynia, consistent with a peripheral site of action. These findings suggest DAGL- β inhibition reverses the negative sensori-discriminative state often associated with inflammatory pain.

TOLERANCE TO THE RATE-DECREASING EFFECTS OF CP55,940 DEVELOPS IN MURINE INTRACRANIAL SELF-STIMULATION. T. W. Grim, J. M. Weibelhaus, S. S. Negus & A. H. Lichtman, Dept. of Pharmacology & Toxicology, Virginia Commonwealth University, Richmond, VA 23298. Although not currently abused, CP55,940 is a synthetic cannabinoid that possesses many similarities to abused "non-classical" cannabinoids such as CP47,497 and its C8 homolog in terms of increased potency and efficacy relative to Δ^9 -tetrahydrocannabinol (THC). Thus CP55,940 was selected as an archetypal synthetic cannabinoid to probe the effects of acute and chronic effects of cannabinoids on operant responding for intracranial self-stimulation (ICSS) of the medial forebrain bundle in C57BL6/J mice. Acutely, CP55,940 (0.3-1.0 mg/kg) produced a dose-related significant depression of operant responding for ICSS (ED_{50} value (95% confidence limits) = 0.18 (0.12-0.26) mg/kg). The CB1 receptor antagonist, rimonabant (3.0-10.0 mg/kg; s.c.), significantly attenuated the rate-decreasing effects of CP55,940, indicating CB1 receptor mediation. Repeated administration of CP55,940 (0.3 mg/kg, s.c., q.d. for 7 days) resulted in a significant attenuation to the drug's rate-decreasing effects, indicative of tolerance. There were no measurable spontaneous withdrawal effects for CP55,940 under these experimental conditions. Collectively, these data suggest that the abuse liability of synthetic cannabinoids occurs through a distinct mechanism from other drugs of abuse (e.g., cocaine) that act through the classic mesolimbic pathway. Nevertheless, operant responding for ICSS represents a novel approach to investigate the impact of acute and chronic administration of cannabinoids on reward-mediated behavior.

DISCOVERY OF SMALL MOLECULE INHIBITORS OF PSAA, A POTENTIAL TARGET FOR *STREPTOCOCCUS PNEUMONIAE*. Ahmad J. Obaidullah¹, Hardik I. Parikah¹, Todd Kitten² & Glen E. Kellogg¹, ¹Department of Medicinal Chemistry, Virginia Commonwealth University, Richmond, VA 23298 & ²Philips Institute for Oral Health Research, Virginia Commonwealth University, Richmond, VA 23298. Due to development of multidrug resistance in *Streptococcus pneumoniae* over the last few

years, research has begun to define new drug targets for pneumonia therapy. Multiple experiments with *Streptococcus pneumoniae* performed by different research groups have identified a lipoprotein PsaA that is important for pneumonia virulence and is also a promising target for pneumonia therapy. PsaA is a high-affinity manganese (Mn) transporter that is required not only for pneumonia virulence, but also for aerobic growth of *Streptococcus pneumoniae* in serum. We have employed computer modeling and computational chemistry to virtually screen a small-molecule database for inhibition of PsaA function by targeting the metal binding pocket, performing receptor-based virtual screening, molecular docking to identify potential inhibitors of PsaA function, and scoring. We have developed an assay for screening compounds, including the use of a PsaA mutant, testing of multiple compounds, and identification of small numbers of compounds that inhibit *Streptococcus pneumoniae* growth at concentrations less than 20 μM . We will next experimentally test the compounds' effect on Mn uptake and their PsaA dependence. Supported by American Heart Association grant 13GRNT16100010 to TK.

VARIATION IN TIMING OF HEART SOUNDS RELATIVE TO ECG EVENTS IN COLLEGE STUDENTS. Harold J. Grau, Department of Molecular Biology & Chemistry, Christopher Newport University, Newport News, VA 23606. Students in our Human Anatomy & Physiology II lab course performed an activity that included simultaneous recordings of electrocardiograms (ECG) and heart sounds. The two basic heart sounds occur first at the closure of the atrioventricular (AV) valves (S1), and then at the closure of the semilunar valves (S2). AV closing occurs just at the beginning of ventricular systole, which follows the depolarization of the ventricles, indicated by the QRS complex of the ECG. The second sound occurs just after the ventricles begin relaxing (diastole), which follows the repolarization indicated by the T wave of the ECG. Data were collected from about 2 dozen subjects, both male and female, between ages of 20 – 22 years old. The delay between peak R – S1 ranged from 9 – 55 ms, with most falling into two “clusters” of 22 – 27 ms, and 41 – 47 ms. The delay between peak T – S2 ranged from 69 – 136 ms, with no obvious “clusters”. The time between peak R – peak T varied from 227 – 309 ms, and correlated with both the peak R – S1 and peak T – S2 delays. However, the peak R – S1 delay does not correlate with the peak T – S2 delay. The time between the two sounds (S1 – S2) varied from 307 – 382 ms, and correlates positively with the ECG correlates of systole (peak R – peak T).

QSAR STUDY OF 4-SUBSTITUTED METHCATHINONE ANALOGS AT DAT AND SERT. F. T. Sakloth¹, J. Partilla², R. Kolanos¹, M. Barnier¹, R. Vekariya¹, P. D. Mosier¹, M. H. Baumann², & R. A. Glennon¹, ¹Department of Medicinal Chemistry, Virginia Comm. Univ., Richmond, VA 23298 & ²National Institutes of Health, National Institute of Drug Abuse, Baltimore, MD 21224. Methcathinone (MCAT) analogs are a popular class of designer drugs and, due to their abuse potential, about 15 are now controlled as US Schedule I drugs. MCAT and its simple 4-substituted analogs act primarily as dopamine and/or serotonin releasing agents in release assays using rat synaptosomes. The goals of this project were: 1) to perform QSAR (quantitative structure-activity relationship) studies of 4-substituted MCAT analogs (n=6) to determine whether action at the dopamine and serotonin transporters (DAT and SERT, respectively) and selectivity might be explained on the basis of their steric

properties, and 2) to construct homology models of the human DAT and SERT using the crystal structure of *Drosophila melanogaster* followed by docking studies to identify how steric character might be explained on the basis of interactions with the two transporters. QSAR studies indicated that increasing length/volume of the 4-position substituent is favorable for SERT action whereas the opposite is favored for DAT. Selectivity for SERT over DAT was found to be highly correlated with volume, length and maximum width ($r > 0.9$). Modeling studies indicated an unfavorable interaction at hDAT, which was not seen at hSERT, in the binding pocket associated with the 4-position substituent. These differences in the binding pockets of the two transporters could explain differences in selectivity. 4-Position steric factors seemingly regulate DAT/SERT selectivity. [Supported by DA DA033930.]

QUANTIFICATION OF HUMAN OSTEOPONTIN, A CANCER BIOMARKER PROTEIN, FROM PLASMA BY MICROFLOW LC-MS/MS. M. Faria¹, M. S. Halquist¹, M. Yuan², W. Mylott Jr.², R. G. Jenkins², & H. Thomas Karnes¹, ¹Virginia Comm. Univ., Richmond, VA 23298, ²PPD Inc, Richmond, VA 23230. Human osteopontin is a secreted plasma protein which is elevated in various cancers and is indicative of poor prognosis. It mainly operates through its integrin binding site ¹⁵⁹RGDSVVYGLR. A method was developed and validated for quantitative measurement of human osteopontin from plasma using microflow LC-MS/MS. *In silico* studies were carried out using online Protein Prospector software and Basic Local Alignment Search Tool to obtain a signature peptide. An immunoaffinity coupled LC-MS/MS method was developed. The applicability of the validated method was demonstrated by quantification of OPN from plasma samples obtained from 10 healthy individuals and 10 breast cancer patients. A biologically relevant tryptic peptide 'GDSVVYGLR' which is unique to human osteopontin was identified and used as a signature peptide for this method. The method was validated over a linear range of 25-600 ng/mL for human osteopontin. The plasma OPN concentrations in healthy individuals ranged from 38-85 ng/ml with a mean concentration of 55±15 ng/ml. A 2-12 fold increase in osteopontin concentrations, ranging from 85-637 ng/ml, were seen in breast cancer patient samples. In conclusion, microflow LC-MS/MS can be used for accurate and precise quantitative bioanalysis of proteins. This validated LC-MS/MS method can be used in clinical settings for measuring osteopontin levels from plasma.

QUINAZOLINES AS NOVEL HUMAN OCT3 INHIBITORS: STRUCTURE ACTIVITY STUDIES. Malaika D. Argade¹, Xiaolei Pan², Kavita A. Iyer¹, Douglas Sweet² & Małgorzata Dukat¹, ¹Department of Medicinal Chemistry, & ²Department of Pharmaceutics, Virginia Commonwealth University, Richmond VA 23298. Globally, depression affects 20% of the population with half being refractory to current therapies. Depression is associated with low synaptic levels of the neurotransmitters serotonin (5-HT) and/or norepinephrine (NE) regulated by high-affinity, low-capacity transporters, SERT and/or NET (uptake-1). Recently, low-affinity, high-capacity transporters, Organic Cation Transporters (OCT1-3; uptake-2) have been identified as an alternative mechanism in regulation of synaptic 5-HT and NE levels. Previously, we reported that 2-amino-6-chlorodihydroquinazoline (A6CDQ) showed antidepressant-like effects in the mouse tail suspension test. A6CDQ lacks affinity at SERT ($K_i > 10,000$ nM), but inhibits hOCT3 ($IC_{50} = 3.9$ μM). To determine whether the 6-chloro group exerts its

effect via its electronic or lipophilic character, we synthesized the 6-methyl analog (A6MDQ) that bears an electron-donating group as opposed to the electron-withdrawing chloro group. Methyl and chloro groups possess similar lipophilic (i.e., $\pi = 0.56$; 0.71 , respectively), but opposite electronic character. A6MDQ inhibited hOCT3 expressed in HEK293 cells ($IC_{50} = 1.6 \mu\text{M}$). This suggests that the lipophilic character of the substituent plays an important role in the inhibitory actions of hOCTs. We generated the first 3-D graphics model of hOCT3 using the inorganic phosphate transporter (PDB:4J05) as a template. The molecular modeling studies agreed with the experimental results obtained since the 6-methyl analog utilizes a similar binding mode as the 6-chloro analog, thereby validating the model.

QSAR AND MOLECULAR MODELING STUDIES ON ARYLGUANIDINES AS $\alpha 7$ nAChR NAMs. O. I. Alwassil¹, S. Khatri², M. K. Schulte² & M. Dukat¹, ¹Department of Medicinal Chemistry, Virginia Commonwealth University, Richmond, VA 23298, ²Department of Pharmaceutical Sciences, University of Sciences, Philadelphia, PA 19104. There is ample literature evidence that $\alpha 7$ neuronal nicotinic acetylcholine receptors (nAChRs) might play a role in the treatment of Alzheimer's disease. Previously we reported that *meta*-chlorophenylguanidine (*m*CPG; 1) and its *N*-methyl analog 2, developed in our laboratory, exert negative allosteric modulator (NAM) actions at $\alpha 7$ nAChRs expressed in HEK cells ($IC_{50} = 8$ and $1.3 \mu\text{M}$, respectively). Initial SAR and 3D graphics models suggested different binding modes. Thus, two series of analogs ($n = 16$) based on 1 and 2 were synthesized and evaluated for functional activity in two-electrode voltage clamp assays using frog oocyte-expressed $\alpha 7$ nAChRs (IC_{50} value range = $21 - 118 \mu\text{M}$; $12 - 125 \mu\text{M}$, respectively). Quantitative structure-activity relationship (QSAR) studies support our initial findings that the two series of NAMs interact in a different manner at $\alpha 7$ nAChRs because parallel structural changes did not result in parallel shifts of activity and there is a lack of significant correlation between the activities of the two series ($r = 0.474$). Furthermore, the studies indicated that electronic ($r = 0.820$) properties might be important at the *meta*-position of 1 analogs and shape (B_1 ; $r = 0.927$) for the 2 series for their NAM actions, and support our proposed 3D graphics model. Supported in part by the Virginia Center on Aging (Award No. 12-2).

DECONSTRUCTION OF RISPERIDONE – HOW FAR IS TOO FAR? S. A. Gaitonde¹, J. Younkin², D. E. Logothetis², R. A. Glennon¹ & M. Dukat¹, ¹Departments of Medicinal Chemistry and ²Physiology & Biophysics, VCU, Richmond, VA 23289. Ketanserin (competitive antagonist) and the antipsychotic agent risperidone (an inverse agonist), though structurally similar, differ in their functional action at 5-HT_{2A} receptors. This study probes this disparity and attempts to envisage the minimal structural features that confer risperidone with its action. The structure of risperidone was deconstructed to give 4 analogs: 6-fluoro-3-(piperidin-4-yl)benzoxazole (FBP), 6-fluoro-3-(1-methyl-piperidin-4-yl)benzoxazole (FBPM), 4-[(4-(6-fluorobenzoxazol-3-yl)piperidin-1-yl)-1-(piperidin-1-yl)]butan-1-one (FBPC) and 6-fluoro-3-[(1-(4-piperidin-1-yl)butyl)piperidin-4-yl]benzoxazole (FBPS). The analogs were synthesized and functional action examined using two-electrode voltage clamp techniques (*Xenopus* oocytes expressing 5-HT_{2A} receptors and Kir3.4 channels). FBPC and FBPS were partial agonists (60% and 62% activity compared to 5-HT,

respectively) whereas FBP and FBPM were competitive antagonists (74% and 100% inhibition of 5-HT activity, respectively). A 3D-graphics model of the h5-HT_{2A} receptor using the crystal structure of the h5-HT_{2B} receptor (PDB 4IB4) as template showed that ketanserin and risperidone differ in their binding mode at TM5 in forming hydrogen bonds with Ser239 and Ser242, respectively, and that risperidone and its analogs displayed similar binding modes and form a bidentate interaction with Ser159 (TM3); ketanserin did not. Based on the modeling results, it can be concluded that, at present, our model cannot distinguish between the observed functional actions. But, inverse agonist action of FBP/FBPM remains to be established.

AN ASSOCIATION BETWEEN THE DEPRESSION AND SYSTEMIC INFLAMMATION IN NHANES POPULATIONS. Rachel Smith^{1,2}, Maria Stepanova^{1,2}, Ancha Baranova^{1,2}, & Aybike Biredinc^{1,2}, ¹Betty and Guy Beatty Center for Integrated Research, Inova Health System, Falls Church, VA 22042 and Center for the Study of Chronic Metabolic Diseases, George Mason University, Fairfax, VA 22030. A population-based study was conducted to determine the association of individuals with chronic diseases and depression from five cycles of the National Health and Nutrition Examination Survey (NHANES) data. The presence of depression was determined from a depression screener questionnaire, while the presence of chronic diseases were gathered from medical conditions questionnaires (3,751 and 26,225 subjects respectfully). Interestingly, individuals with depression and those with multimorbidity had the same statistically significant increased inflammatory markers as assessed by HOMA scores, C-reactive proteins, white blood cell counts, and percentages of segmented neutrophils and glycohemoglobin ($p < 0.05$). Additionally, both had statistically significant decreased levels of HDL-cholesterol, lymphocyte percentages, and monocyte percentages ($p < 0.05$). The increased inflammatory markers suggest that low-grade chronic inflammation may be the connection between depression and chronic diseases in these individuals. Examining these inflammatory markers may be used as predictors in the treatment of patients.

MITOCHONDRIAL HAPLOGROUP ASSESSMENT IN OBESE PATIENTS WITH NAFLD. Kianoush Jeiran¹, Rohini Mehta², Ancha V. Baranova¹, & Zobair Younossi², ¹School of Systems Biology, George Mason University, Manassas, VA 20110 and ²Claude Moore Health Education and Research Center, Inova Fairfax Medical Campus, Falls Church, VA 22042. Liver disease is considered a significant health problem worldwide nowadays. Among distinct disease, non-alcoholic fatty liver disease (NAFLD), which is widely considered the hepatic manifestation of the metabolic syndrome, is a complex multifactorial disease trait where environment and genetic variations interact to determine the wide spectrum of disease progression. One of the key challenges is to predict the progression of NAFLD; mitochondrial genotyping may serve as an indicator of increased susceptibility to progressive NAFLD. In this study the association of sequence variations (haplogroups and indels) in control loop of mitochondria within 86 patients with biopsy-proven NAFLD and metabolic syndrome was investigated. After extracting DNA from whole blood cells and amplifying control loops by PCR and sequencing the control region; samples have been categorized within 11 haplogroups (H, I, J, K, L, M, T, U, V, W, and X). Liver biopsy pathology has revealed that 64 (74.4%) patients diagnosed with NAFLD and 22 (25.6%) patients had

normal biopsy. Moreover, 80.8% of patients with Non-L mtDNA haplogroups progress to NAFLD, but only 38.8% of patients with L haplogroup diagnosed with NAFLD which proves that obese patients with L haplogroup are much less susceptible to develop non-alcoholic fatty liver comparing to other mtDNA genotypes.

Posters

REGULATION OF SEROTONIN TRANSPORT FUNCTION AND CELL SURFACE EXPRESSION BY BRAIN-DERIVED NEUROTROPHIC FACTOR. J. Rajamanickam, K. N. Naidu, L. Jayanthi, & S. Ramamoorthy, Dept. of Pharm. & Tox., VCU, Richmond VA 23298. The serotonin (5-HT) transporter (SERT) maintains the level of serotonin in the synaptic cleft by reuptaking released 5-HT into presynaptic terminals. Dysfunction of SERT leads to several psychiatric disorders like autism, depression, obsessive-compulsive disorder, and addiction. Selective serotonin reuptake inhibitors (SSRIs) are specifically target SERT and inhibit 5-HT reuptake in the treatment of psychiatric disorders. A member of neurotrophin family of proteins, brain derived neurotrophic factor (BDNF), which binds to Tropomyosine-related kinase B receptor (TrkB), reduces SSRI mediated inhibition of 5-HT reuptake by increasing SERT activity. Current study was aimed to understand the regulation of activity and expression of SERT by BDNF and TrkB. BDNF treatment of HEK 293 cells co-expressing SERT and TrkB increased the SERT activity parallel with increased surface level of SERT protein. Kinetic assay revealed increase in Vmax of SERT following BDNF treatment. BDNF treatment also increased the phosphorylation of endogenous protein kinases ERK1/2 and Akt in the HEK 293 cells. Treatment of HEK 293 cells with the inhibitors of ERK1/2 (PD98059) and Akt (Akt inhibitor X – AktX) reduced the 5-HT uptake in a concentration dependent manner. BDNF treatment reversed the inhibitory effect of PD98059 but not of AktX on SERT activity. This result indicates that BDNF may stimulate the 5-HT uptake indirectly through increasing the phosphorylation of ERK1/2. The overall observations suggest that BDNF regulates surface expression and function of the serotonin transporters.

COMPARISON OF THE COGNITIVE EFFECTS OF THE NMDA ANTAGONIST KETAMINE IN ADOLESCENT AND ADULT RATS. M. P. George and K. L. Nicholson, Department of Pharmacology and Toxicology, Virginia Commonwealth University, Richmond, VA 23298. Ketamine has been subject to growing rates of abuse worldwide, particularly in adolescent populations. Previous studies in the laboratory have shown differences in abuse-related behavioral effects of ketamine in rats based on age. The current project extends this investigation by comparing the effects of ketamine on memory in a novel object recognition procedure in male Sprague-Dawley rats. Activity in a 17 in. x17 in. experimental chamber was recorded and analyzed using ANYmaze software. Rats underwent two 6-min training periods, separated by 1 hour, during which two like objects were present in the chamber. After an hour, one object was replaced with a novel item and rats returned to the chamber for a 6-min testing period. Time spent in the two zones when the like items were present was compared to the times when one item was replaced with a novel object. Baseline performance was determined across three age groups: adolescent (PND34-44), young adult (PND70-90) and older adult (PND150-180). Subsequently ketamine and cocaine

were tested in adult rats for disruption of memory retrieval. In baseline tests, adolescents spent more time in the novel object zone, however due to a high level of variability, the effect was not significant. Both young and older adult rats demonstrated evidence of learning with significantly more time spent with the novel object. Cocaine and ketamine produced dose-dependent disruption of memory retrieval in both groups of adult rats. The effect of additional habituation and training in adolescent subjects is being investigated prior to drug testing in this age group.

DEVELOPMENT OF NATURAL CYCLIC PEPTIDE INHIBITORS OF XRCC4/XLF INTERACTION FOR RADIO-SENSITIZATION OF BREAST TUMOR CELLS. M. Al Mohaini¹, K. Akopiants¹, E. R. White², M. C. T. Hartman^{2,3}, & L. F. Povirk^{1,3}, ¹Dept. of Pharm. & Tox., ²Dept. of Chemistry & ³Massey Cancer Center, VCU, Richmond, VA 23298. Breast cancer is the second leading cause of cancer death in women, and the standard treatment is ionizing radiation combined with surgery and chemotherapy. Cell death after exposure to radiation results largely from DNA double-strand breaks. Previous studies showed that many breast tumor cells depend mainly on non-homologous end joining (NHEJ) for repairing induced DNA damage. XRCC4 and XLF are two essential interacting proteins in the NHEJ process. A single mutation on the XLF-binding interface of XRCC4 at M61, F106, E55 or D58 has been shown to disrupt its interaction with XLF, thus inhibiting NHEJ. Therefore, it is proposed that small natural cyclic peptides that bind to the XLF interface of XRCC4 near M61 and F106 can be identified through an mRNA display *in vitro* selection, and these peptides will inhibit NHEJ and thereby radiosensitize breast tumor cells. Using mRNA display, we created a library of a trillion unique cyclic mRNA-peptide fusions. After seven rounds of *in vitro* selection, the eluted fusions were cloned and sequenced. Results showed homology of sequences of five main families (Pep 7.1-7.5), and we have synthesized representative peptides of these families. Fluorescence polarization studies showed binding affinities of Pep 7.1 and Pep 7.2 to XRCC4¹⁵⁷ protein with K_D of 16.78 and 18.77, respectively. Furthermore, Pep 7.1 and Pep 7.2 were able to inhibit the rejoining of a simple restriction-cut plasmid in an extract of patient derived XLF cells supplemented with XLF protein.

THE ROLE OF P38 MITOGEN-ACTIVATED PROTEIN KINASE MEDIATED NOREPINEPHRINE TRANSPORTER REGULATION IN COCAINE-INDUCED BEHAVIOR. P. Mannangatti, K. Narasimha Naidu, M. I. Damaj, S. Ramamoorthy, & L. D. Jayanthi, Dept. of Pharm & Tox., VCU, Richmond, VA 23298. The noradrenergic and p38 mitogen activated protein kinase (p38 MAPK) systems have been implicated in behavioural effects of cocaine. The present experiments were designed to probe functional interactions between norepinephrine transporter NET and p38 MAPK by testing the role of p38 MAPK-mediated NET regulation in cocaine-mediated behaviours. In Experiment 1, mice pre-exposed to i.p. cocaine (15 mg·kg⁻¹, day 1; 30 mg·kg⁻¹ days 2-4; and no drug, days 5-8) or saline were pretreated with SB203580 (50-100 µg·kg⁻¹, i.p), before testing for locomotor sensitization in response to cocaine challenge (15 mg·kg⁻¹, day 9). In Experiment 2, mice were subjected to unbiased cocaine CPP where mice received 10 mg·kg⁻¹, i.p cocaine for 4 days and on day five received vehicle or SB2035804-[5-(4-Fluorophenyl)-2-[4-(methylsulfonyl)phenyl]-1H-imidazol-4-yl]pyridine (50-100 µg·kg⁻¹, i.p), and tested for

preference scores. In Experiment 3, after behavioural testing, NE transport function and NET expression levels along with phospho-p38 MAPK levels were measured in the prefrontal cortex (PFC) and nucleus accumbens (NAc) of the mice. Pretreatment with SB203580 selectively interfered with cocaine-induced locomotor sensitization and CPP. In addition, SB203580 pretreatment blocked cocaine-induced p38 MAPK activation and the upregulation of NE transport and NET expression in the PFC and NAc brain regions of the mice. These findings reveal a mediating role for p38 MAPK-mediated NE transmission in cocaine-related behaviours.

THE DUAL FAAH/MAGL INHIBITOR SA-57 SERVES AS A DISCRIMINATIVE STIMULUS. R. Owens¹, B. Cravatt², & A. Lichtman¹, ¹Dept. of Pharm. & Tox., VCU, Richmond, VA 23298, ²The Skaggs Institute, & TSRI, La Jolla, CA 92037. The drug discrimination paradigm is a very sensitive assay to infer interoceptive effects of a variety of psychoactive drugs, including cannabinoids. The phytocannabinoid Δ^9 -tetrahydrocannabinol (THC) serves as a discriminative stimulus in rodents via cannabinoid receptor type one (CB₁) mechanism of action. Simultaneous inhibition of the endocannabinoid degradative enzyme fatty acid amide hydrolase (FAAH) and monoacylglycerol lipase (MAGL) produces THC-like effects in this paradigm. More recently, the development of the dual FAAH/MAGL inhibitor SA-57, which is more potent at inhibiting FAAH than MAGL, provides a useful tool to investigate the impact of differentially inhibiting these endocannabinoid degradative enzymes. Mice were trained to discriminate between the potent synthetic cannabinoid CP55,940 (0.1 mg/kg) and vehicle in a nose-poke discrimination task (ED_{50} (95% confidence interval) = 0.34 0.058 - 1.99) mg/kg. SA-57 (10 mg/kg) fully substituted for CP55,940. The discriminative stimulus effects of both CP55,940 and SA-57 were completely antagonized by 3 mg/kg rimonabant, indicating a CB₁ receptor mechanism of action. These findings suggest that SA-57 produces a similar interoceptive stimulus as CP55,940. Importantly, mice learned to discriminate between SA-57 and vehicle in a dose-related fashion (ED_{50} value = 4.49 mg/kg, 95% CI 3.77 – 5.35 mg/kg). This study represents the first demonstration that complete blockade of the primary endocannabinoid degradative enzymes FAAH and MAGL serves as a reliable discriminative stimulus.

ADIPONECTIN EXPRESSION IN PATIENTS SUFFERING FROM METABOLIC DISORDERS. Kameron M. Tavakolian & Michael Estep, The Beatty Liver & Obesity Research Program, Inova, Falls Church, VA 22042, School of Systems Biology, College of Science, George Mason University, Fairfax, VA 22030. Adiponectin is an essential adipokine released from adipose tissue with antiatherogenic, anti-diabetic, and anti-inflammatory properties. Individuals with fatty liver disease show reduced circulating levels of adiponectin independent of body mass index, which is inversely correlated to the degree of steatosis and inflammation. The aim of this study was to measure the expression of adiponectin and its receptor, AdipoR1, in visceral adipose tissue and its relation to hepatic fibrosis. RNA was extracted from visceral adipose tissue from a selected cohort of 19 morbidly obese patients using a BIORAD kit. The iScript cDNA synthesis kit was used and RT-PCR was performed with AdipoQ and AdipoR1 primers, along with GAPDH and RP2 as endogenous controls. Primers for the study were designed on NCBI with stringent criteria such that each primer crossed

an intron and the deltaG setting minimized hairpin formation and primer dimerization. No correlation between adiponectin expression and severity of fibrosis was observed, although a larger sample size would be needed to determine whether adiponectin plays a role in fibrosis.

HYDROXYMETHYLATION AND ALZHEIMER'S DISEASE: A MAP OF DYNAMIC REGIONS IN THE DISEASED STATE. R. H. Haraf, M. Baker, J. Davy, A. Hazy, D. Harrison, N. Taher, and G. Isaacs, Dept. of Biology, Liberty University, Lynchburg VA 24502. Alzheimer's Disease (AD) is a neurodegenerative disorder that is currently responsible for approximately 500,000 deaths per year. Because less than 5% of cases result from a genetic mutation, epigenetic factors are being considered as a potential cause for the majority of AD patients. Hydroxymethylation, a recently discovered epigenetic modification to cytosine, has been found to be especially prevalent and active in the brain. Furthermore, it has been linked to neurogenesis, neural differentiation, and normal neurological function. For this reason, understanding the exact role of hydroxymethylation in the brain may shed light on the cause and pathogenesis of AD, as well as other neurological disorders. This study sought to map the distribution of hydroxymethylation in the brain of a healthy mouse and a mouse displaying AD-like characteristics. Using a double transgenic murine model that developed beta-Amyloid plaques in the brain, hippocampal DNA was tested for the presence of hydroxymethylation in the promoter regions of genes. Changes in hydroxymethylation distribution between the healthy and AD mice were mapped, allowing the determination of 206 specific genes of interest that may be dysregulated in Alzheimer's Disease.

THE EFFECTS OF DOXYCYCLINE-INDUCED HIV-1 TAT PROTEIN EXPRESSION IN MICE ON THE STARTLE REFLEX AND ITS PREPULSE INHIBITION. R. M. Enga, K. F. Hauser, & P. M. Beardsley, Department of Pharmacology & Toxicology, Virginia Commonwealth University, Richmond, VA 23298. Motor and behavioral deficits, including deficits of attention, are present in HIV-associated neurocognitive disorders (HAND) that may, in part, be attributable to HIV-1 Tat protein expression. Sensorimotor gating is linked to attention, and can be measured by the acoustic startle response and its prepulse inhibition (PPI). To determine whether HIV Tat contributes to impaired sensorimotor gating, we tested doxycycline (DOX)-inducible Tat transgenic mice and C57BL/6J (background strain) mice for baseline PPI to a 119 dB startle pulse with 73, 77, and 85 dB prepulses. The mice were re-evaluated 2, 9, and 16-d post-DOX administration. Locomotor activity was also assessed in a 2-h test session in naïve Tat transgenic mice. Baseline PPI was not significantly different between groups; however, PPI significantly decreased in Tat(+)/DOX after 2, 9, and 16-d of DOX administration ($p \leq 0.05$). PPI was unaltered in DOX-treated Tat(-) and C57BL/6J mice, indicating a lack of nonspecific DOX effects. PPI was also unaltered in untreated Tat(+), Tat(-), and C57BL/6J mice. In locomotor activity tests, baseline total distance traveled was not significantly different between groups, however after DOX administration, Tat(+)/DOX mice traveled significantly less than Tat(-)/DOX mice ($p \leq 0.05$). These results suggest that HIV Tat induction caused sensorimotor gating and locomotor deficits in mice, which may serve

as predictive correlates of some aspects of the behavioral and motor pathology associated with HAND.

MITOCHONDRIAL DYSFUNCTION IN STREPTOZOTOCIN ALZHEIMER'S DISEASE MODEL. Essie S. Komla¹, Ann C. Rice¹, Paula I. Moreira², & James P. Bennett Jr.¹, ¹Parkinson's and Movement Disorders Center, Virginia Commonwealth University, Richmond, VA 23298 & ²Center for Neuroscience and Cell Biology of Coimbra, University of Coimbra, 3030 Coimbra, Portugal. Alzheimer's disease (AD) is the most common type of dementia found in the adult population. The disease is characterized by death or malfunction of neurons leading to memory loss, cognitive decline, and increased level of amyloid beta peptides and neurofibrillary tangles. Some researchers suggest AD is an "insulin-resistant brain state" disorder and the use of intracerebroventricular (icv) injection of streptozotocin (STZ) in rats is emerging as an experimental model for the disease. We hypothesize we will observe increased expression of mitochondrial biogenesis markers in the icvSTZ rats as a compensatory response to decreased metabolism. Using laser capture microdissection (LCM) to collect multiple groups of 20 hippocampal pyramidal neurons, we determined the mitochondrial DNA (mtDNA) copy numbers from 5 icvSTZ rats and 4 control (CTL) cases using quantitative polymerase chain reaction (qPCR). We found no significant difference between the mtDNA copy numbers of the STZ samples compared to the control. However, these preliminary data show a trend toward increased mtDNA copy numbers in STZ treated brain which is one of the biomarkers of mitochondrial biogenesis. In conclusion, our preliminary data show an increase in mitochondrial gene copy numbers as the cells try to compensate for the degeneration of the neurons; thereby, increasing mitochondrial synthesis.

THERAPEUTIC EFFICACY OF COMBINATION OF MTOR INHIBITORS AND AMPK ACTIVATORS IN NON-SMALL CELL LUNG CANCER. Grinal M. Corriea & Richard G. Moran, Dept. of Pharmacology & Toxicology, Richmond, VA 23298. Pemetrexed (PTX), an antifolate drug, has been approved by the FDA for first line therapy of mesothelioma and non-small cell lung cancer. In addition to its primary site of action on thymidylate synthase, PTX also inhibits the second folate-dependent enzyme of purine biosynthesis, aminoimidazolecarboxamide ribonucleotide formyltransferase (AICART). Accumulation of AICART substrate, ZMP in PTX-inhibited cancer cells leads to downstream activation of AMP-activated protein kinase (AMPK) with subsequent inhibition of mammalian target of rapamycin (mTOR) and hyperphosphorylation of its downstream targets responsible for protein synthesis and cell proliferation. Inhibitors of mTORC1 like Rapamycin and its analogs have only partial effect, as they do not inhibit mTORC2, which phosphorylates Akt subsequently relieving the inhibition of mTORC1 and leading to poor cytotoxicity. AZD8055 is an ATP-competitive inhibitor of mTOR kinase and potently inhibits both mTORC1 and mTORC2. Our data shows that AMPK activation via PTX-mediated AICART inhibition in combination with direct mTOR inhibition by AZD8055 has a synergistic interaction on the proliferation of cells in culture. Activators of AMPK enhance the effects of direct inhibitors of mTOR and vice-versa and these effects are mediated via combined effects on mTORC1. Taken together, it is a promising therapeutic strategy.

SENSITIZATION OF NON-SMALL CELL LUNG CANCER (NSCLC) TO RADIATION BY VITAMIN D BY A CYTOSTATIC FORM OF AUTOPHAGY. K. Sharma, R. W. Goehe, X. Di, S. Torti, F. Torti & D. A. Gewirtz, Dept. of Pharm. & Tox., VCU, Richmond, VA 23298. The standard of care for unresectable lung cancer is chemoradiation. However, therapeutic options are limited and patients are rarely cured. In A549 and H460 non-small cell lung cancer (NSCLC) cells, 1,25-D₃ (the hormonally active form of vitamin D) and EB 1089 prolonged the growth arrest induced by radiation alone and suppressed proliferative recovery, which translated to a significant reduction in clonogenic survival. In H838 or H358 NSCLC cells, which lack the vitamin D receptor or functional p53, respectively, 1,25-D₃ failed to modify the extent of radiation-induced growth arrest. Sensitization to radiation in H1299 NSCLC cells was evident only when p53 was induced in otherwise p53 null H1299 NSCLC cells. Sensitization was not associated with increased DNA damage or an increase in apoptosis, necrosis or senescence. Instead sensitization appeared to be a consequence of the conversion of the cytoprotective autophagy induced by radiation alone to a novel cytostatic form of autophagy by the combination of vitamin D with radiation. While both pharmacological and genetic suppression of autophagy or inhibition of AMPK phosphorylation sensitized the NSCLC cells to radiation alone, inhibition of the cytostatic autophagy induced by the combination treatment reversed sensitization. Taken together, these studies have identified a unique cytostatic function of autophagy that appears to be mediated by the vitamin D receptor, p53 and AMPK in the promotion of an enhanced response to radiation by 1,25-D₃ and EB 1089 in NSCLC.

CHRONIC MORPHINE ENHANCES NICOTINE RESPONSES IN SINGLE MOUSE ENTERIC NEURONS. Aravind R. Gade, William L. Dewey and Hamid I. Akbarali, Department of Pharmacology & Toxicology, Virginia Commonwealth University, Richmond, VA 23298. Opioids are excellent pain relievers. A major side-effect of chronic opioid treatment is constipation whereas withdrawal following chronic exposure leads to diarrhea and increased gastrointestinal motility. These effects of chronic opioids are mediated by mu opioid receptors expressed on enteric neurons. Previous in-vitro studies have shown that chronic opioids enhance sensitivity to nicotinic receptor agonists. In this study we examined the sensitivity to nicotine following short (10 minutes) and long-term exposure (16-20 hours) to morphine in isolated mouse single enteric neurons using patch-clamp techniques. Nicotine (1mM) induced inward currents from holding potential of -60 mV that were significantly increased in neurons exposed for long-term but not short-term to morphine. However a significant shift was not seen in the EC (50) values in all neurons. The maximal current densities induced by 1mM nicotine were 168 ± 29 pA/pF, 135 ± 31 and 296 ± 51 pA/pF in control, short-term and long-term morphine treated cells respectively, and the EC (50) values were 44 ± 17 μ M (n = 9), 42 ± 14 (n = 5) and 26 ± 7 μ M (n = 11) in the same order. Shift in the maximal currents but not in the EC (50) values suggests a shift in the efficacy but not potency of nicotine after long term exposure to morphine. Studying the mechanisms involved in this enhancement is hypothesized to give a better insight into methods for treating opioid dependent and withdrawal symptoms.