

Medical Science

HIGH DIETARY SALT AND FRUCTOSE INCREASE NFAT5 EXPRESSION IN THE KIDNEY AND LIVER OF SPRAGUE DAWLEY RATS. B. Herman¹, K. Ferguson¹, J. Halterman^{1, 2} & J. Fernandez², ¹Dept. of Biology and ²MA in Biomedicine Program, Eastern Mennonite University. In the current diet of an average American, there is an increasing amount of both salt and high fructose corn syrup. Individuals with a higher sensitivity to salt or fructose can develop hypertension and metabolic syndrome. This study aimed to determine how the consumption of a high salt diet or a high fructose diet altered tissue-specific expression of the NFAT5 gene. A total of 50 Sprague Dawley rats were put either on a control diet of 0.25% NaCl and 6% fructose, a 4% NaCl diet, 8% NaCl diet, or 64% fructose diet over the course of 8 weeks. After 8 weeks, 16 different body tissue samples were harvested. RNA was then purified from the samples, reverse transcription was used to convert the purified RNA into DNA, and DNA samples were then run under real-time PCR in order to measure expression of the NFAT5 gene. The results showed an increase in NFAT5 expression in the kidney medulla under a 4% NaCl diet and in the kidney cortex under a 64% fructose diet. The results also displayed an increase in NFAT5 expression in the liver under a 64% fructose diet and 8% NaCl diet. From this study it can be concluded that NFAT5 is differentially expressed in different tissues in response to various diets. This study was funded by The Thomas F. and Kate Miller Jeffress Memorial Trust, Bank of America, Trustee.

ROLE OF THE GASTROINTESTINAL MICROBIOME IN OPIOID TOLERANCE. R.A. Mischel, M. Kang, W. Dewey, & H.I. Akbarali, Dept. of Pharmacology & Toxicology, Virginia Commonwealth University, Richmond, VA 23298. Gastrointestinal microbial dysbiosis is known to alter physiologic homeostasis and contribute to pathogenesis. Though morphine and other narcotics are the most widely prescribed therapy for moderate to severe pain, they have been noted to alter microbial composition and promote bacterial translocation to other tissues. Translocated microbes may then modulate local cell signaling and gene expression. One of the most immediately vulnerable compartments following bacterial dissemination is the intestinal wall, containing many terminal processes of extrinsic primary afferent neurons (EPANs) from dorsal root ganglia (DRG). These neurons play an integral role in analgesic tolerance, a major limiting factor of clinical narcotic use. Despite this, the impact of intestinal microbiota on the development of tolerance in these cells has not been well characterized. To this end, we investigated how bacterial depletion via broad-spectrum antibiotic treatment (ABX) impacts nociceptive tolerance with chronic morphine use in mice. We found

that ABX was effective in preventing tolerance in both the tail-immersion and acetic acid stretch assays of behavioral nociception. These findings were recapitulated on a single-cell level in neurons isolated from DRGs; namely, ABX prevented tolerance development to morphine-induced reductions of excitability, as measured by increases of threshold potential in whole-cell current clamp recordings. These findings suggest that gastrointestinal flora play an important role in modulating the pharmacodynamic properties of morphine in mice, and may be a useful target of therapy in man.

CORRELATION OF MOLECULAR MARKERS WITH QUANTIFIED FIBROSIS LEVELS OF NON-NAFLD, NAFLD, AND NASH LIVER SAMPLES. S. Stoddard^{1,2}, Z. D. Goodman³, A. Birerdinc² & A. Baranova^{1,2}, ¹School of Systems Biology, College of Science, George Mason University Fairfax, VA, ²Center for the Study of Chronic Metabolic Diseases, George Mason University, Falls Church, VA, ³Inova Health System, Falls Church, VA. Global prevalence of nonalcoholic fatty liver disease (NAFLD) is currently at 25%, and is expected to increase as a major public health concern due to the current worldwide obesity epidemic. The spectrum of NAFLD includes nonalcoholic steatohepatitis (NASH) and steatohepatic hepatocellular carcinoma (SH-HCC). Not all patients with NAFLD progress to NASH, and the diagnosis and grading of NAFLD/NASH is dependent on the “gold standard” of invasive liver biopsy. Molecular mechanisms behind the development and progression of this disease are poorly understood, and this study attempts to correlate serum biomarkers and adipocyte gene expression with quantified fibrotic liver changes in NAFLD patients. Liver biopsies from obese non-NAFLD, NAFLD, and NASH patients were analyzed via computerized morphometry to quantify levels of steatosis and fibrosis. Preliminary results of immunoassays on serum samples indicate a negative correlation of INF- γ ($p < 0.05$), IL-4 ($p < 0.05$), and G-CSF ($p < 0.01$) with percent collagen of liver biopsies. qPCR of adipose tissue will also be performed for further understanding of this tissue as a driver of the disease process. Correlation of serum biomarker levels with amount of fibrosis as determined by computerized morphometry will be analyzed to assess potential biomarkers for use as a diagnostic and/or prognostic tool.

UNDERSTANDING OMICS PROFILING EXPRESSION BY USING DISTANCE-BASED ANALYSIS AND NETWORK BIOLOGY. T. Cui, J. R. Hamre & A. V. Baranova, School of Systems Biology, George Mason University, Manassas VA. 20110. With large amount of Omics data being generated every day, it's important to link the omics profiling expression to patients who have varying degrees of disorder. In this study, we propose a novel approach integrating distanced-based

analysis and network biology to analyze transcriptomics and proteomics data. We used several datasets including mRNA-Seq, miRNA-Seq and proteomics datasets with different disease states from GEO, TCGA and our collaborator at NIH. Samples from each group were clustered close to the attractor that defines their corresponding space center by global distances. The invasive breast carcinoma mRNA-Seq dataset from TCGA showed a substantial separation between normal/cancer samples. Additionally, the comparison between colon adenocarcinoma miRNA-Seq and mRNA-Seq plot showed a better separation between different disease states from the former, which suggests the holistic miRNA landscape has superior predictive power and can be used as a better diagnostic and prediction tool for reflecting pathophysiological states of human tumors. Lastly, we applied our model to a HDL proteomics dataset that is from 101 patients who have varying degrees of cardiovascular disease. We were able to find four key proteins that may be responsible for the disease severity. In conclusion, using the measurement of Pearson's holistic distances together with network biology analysis, we demonstrated that the omics profiling can be used to reflect sample's regulatory landscapes of overall gene/protein expression signatures, and help us understand the role of diseased-associated genes/proteins in the complex system.

NFAT5 IS DIFFERENTIALLY REGULATED IN THE BRAIN AND BLADDER OF RATS FED A 4% NACL, 8% NACL, AND 64% FRUCTOSE DIET. K. M. Ferguson, J. V. B. Fernandez & J. A. Halterman, Dept. of Biology, Eastern Mennonite University, Harrisonburg VA 22802. This research investigates the effects of a 4% NaCl, 8% NaCl, 64% fructose, and control diet on the expression of Nuclear Factor of Activated T-Cells 5 (NFAT5) in rat tissues. In human health, diets low in salt lower the risk of cardiovascular disorders and diets low in fructose lower the risk of metabolic disorders. However, "low in salt/fructose" is a relative term because studies have demonstrated individual differential responses to the same diets. In this study, the effects of salt/fructose diets on the expression of NFAT5 were examined in the bladder and brain of Sprague Dawley rats. This was done by purifying and quantifying RNA, creating cDNA, and quantifying NFAT5 expression by real-time PCR. The bladder tissue showed decreased expression of NFAT5 following consumption of an 8% salt and 64% fructose diet. The brain tissue showed NFAT5 expression is increased following consumption of a 4% salt diet. These results suggest that NFAT5 is differentially regulated in various tissues of the body in response to dietary changes. Further inquiry into the expression of NFAT5 in other tissues is needed in order to obtain a comprehensive understanding of NFAT5 regulation in the body.

Funding source: The Thomas F. and Kate Miller Jeffress Memorial Trust, Bank of America, Trustee.

EXPLORING PHARMACOLOGICAL AND BEHAVIORAL MECHANISMS INVOLVED IN ALCOHOL DEPENDENCE DURING ADOLESCENCE. Rabha M. Younis^{1,2} & Imad Damaj², ¹Dept. of Microbiology & Genetics, ²Dept. of Pharmacology & Toxicology, Virginia Commonwealth University, Richmond, VA, 23219. Alcoholism is a serious illness that is marked by uncontrollable drinking and physical dependence to alcohol. Long-term alcoholism has been linked to many health concerns such as cirrhosis of the liver and cardiovascular disease. Alcohol is one of the most commonly used drugs among adolescent populations. Given that adolescence is a unique developmental stage during which alcohol has long-term effects on future drug-taking behavior; it is essential to understand how early exposure to alcohol during adolescent may affect the abuse liability of the drug later in life. Our studies focus on identifying behavioral mechanisms involved in alcohol dependence during adolescence by using well-established mouse models of alcohol drinking. We hypothesize that exposure to alcohol during early adolescence will increase alcohol intake later in adulthood. We investigated the impact of alcohol drinking in male and female early adolescent C57BL/6J mice using the Drinking in the Dark (DID) model. Our results showed that exposure to alcohol during early adolescence enhanced ethanol intake later in adulthood in the DID paradigm. Our data illustrates that enhanced alcohol intake are affected by the duration and age of exposure. In addition, we conducted behavioral studies to elucidate the mechanisms underlining the relationship between adolescent ethanol exposures and enhance alcohol intake in adult mice. Our results showed that ethanol exposure during adolescence altered the aversive state and enhanced the rewarding properties of ethanol later in adulthood.

A FORMAL EXAMINATION OF TIMING OF HEART SOUNDS RELATIVE TO ECG EVENTS IN COLLEGE STUDENTS. Harold J. Grau & Michaela Miller, Dept. of Molecular Biol. & Chem., Christopher Newport Univ., Newport News, VA 23606. During each cardiac cycle, the first heart sound (S1) is produced after the ventricle begins contracting (enters systole), the pressure from which causes the atrioventricular valve to slam shut. Once the ventricle begins relaxing (ends systole), the semilunar valves close, creating the second heart sound (S2). We recorded electrocardiograms (ECGs) and heart sounds from 29 female (n=17) and male (n=12) college students (all between the ages of 20 – 22 years of age) to see if there were any patterns to variations in the timing of these heart sounds relative to the beginning of systole (the peak R wave on the ECG) and end of systole (peak T wave on the ECG). We also looked at other parameters such as heart

rate, height, weight, and Basal Metabolic Index (BMI) to see if any correlations occurred. There were no significant differences between males and females (although there were differences in the distribution of both heart sound delays). The timing of the heart sounds did not correlate with each other, nor with heart rate, but did show a significant (S1: $P < 0.01$, S2: $P < 0.05$) correlation to the BMI. Heart rate and BMI did not correlate, but heart rate did correlate inversely ($P < 0.05$) with the interval between S1 and S2, which was also inversely correlated significantly with the S1 delay ($P < 0.01$) and with heart rate ($P < 0.05$). A longer S1 delay means that the ventricle takes longer to reach the valve-closure pressure; a longer S2 delay indicates a longer period for the ventricular pressure to fall below aortic pressure. The fact that these are positively correlated with BMI suggests a possible health link that merits further investigation.

EFFECTS OF METHYLENE BLUE ON THE ONSET AND PROGRESSION OF BEHAVIORAL DEFICITS IN A TRANSGENIC MOUSE MODEL OF ALZHEIMER'S DISEASE. H. J. Grau, S. E. Fink, Q. E. Pace, B. C. Genovese, N. Khan, E. Croushore, K. Whitcomb, R. A. Schendzielos, D. A. Mitrano & L. S. Webb, Dept. of Molecular Biol. & Chem., Christopher Newport Univ., Newport News, VA 23606. Alzheimer Disease (AD) is a degenerative form of dementia that is associated with the accumulation of neurofibrillary tangles (tau protein) and amyloid= β (AB) plaque formation. Methylene blue (MB) has been FDA approved for reducing protein aggregations in AD and other diseases. In this study, we used a triple transgenic mouse model of AD (3xTg-AD) to assess any effects of MB on the formation of these protein tangles and deposits development (reported elsewhere) and on spatial learning and memory tasks (reported here), by comparing mice given weekly intraperitoneal injections of MB with those given saline injections (controls). We used the Morris Water Maze (MWM) to test for spatial learning and memory on mice at 3, 4.5, 6, 7.5, 9, 12, 15, and 18 months of age; mice were given 5 days of training (with platform), and then tested (no platform) on day 6. The completed study will have 12 mice for each treatment at each age end-point; the data reported here include results from about half of that total. While the results to date are not conclusive, mice at 6 and 7.5 months of age did perform better at the MWM training (spatial learning) than their saline counterparts. On the test day, which assesses spatial memory, MB treated mice did better at 3, 6, and 15 months of age. Differences between the groups should become more evident once the data are complete.

NEUROPATHOLOGICAL EFFECTS OF METHYLENE BLUE ON THE ONSET AND PROGRESSION OF ALZHEIMER'S DISEASE IN A TRANSGENIC MOUSE MODEL. R. A. Schendzielos¹, S. E.

Fink^{1,2}, Q. E. Pace¹, N. Kahn², B. C. Genovese¹, E. Croushore², K. Witcomb¹, D. Mitrano^{1,2}, L. S. Webb^{1,2}, & H. J. Grau^{1,2}, ¹Dept. of Molecular Biology & Chemistry and ²Program in Neuroscience, Christopher Newport University, Newport News VA 23606. Alzheimer's disease (AD) is a neurodegenerative disease that has been shown to cause neurological changes in the brain, including the development of both amyloid-beta (A β) plaques and neurofibrillary (tau) tangles, as well as impaired cognitive functioning. Past studies have shown that Methylene blue (MB), an FDA approved compound, can reduce the formation of protein aggregates in AD and other diseases. In this study, the 3xTg-AD mouse model, which contains the human transgenes PS1_{m146V}, APP_{Swe}, and Tau_{p301L}, was used to further explore the effect that MB has on the development of AD, specifically on the accumulation of the A β plaques and tau tangles. Weekly intraperitoneal injections of either MB (10 mg/kg) or 0.9% saline were given, starting at 4 weeks and continuing until either 3, 4.5, 6, 7.5, 9, 12, or 15 months of age. At these endpoints, the mice were transcardially perfused with 4% paraformaldehyde/0.1% glutaraldehyde, and the brains were removed and fixed for 72 hours in 4% paraformaldehyde and then cut with a vibrating microtome at 60 μ m. The tissue samples were stained with the clone 6E10 mouse monoclonal antibody for A β plaques and with the p-tau rabbit antibody for the tau tangles. Preliminary qualitative observations of the tissues suggest that the MB-treated mouse brains had less A β and tau accumulation; future work will incorporate quantitative analyses.

ROLE OF VAV2 IN PODOCYTE INFLAMMASOME ACTIVATION AND GLOMERULAR INJURY DURING HYPERHOMOCYSTEINEMIA. S. M. Conley¹, Z. Chen¹, M. Xia¹, T. W. Gehr², K. M. Boini^{1,2} & P.L. Li¹, ¹Dept. of Pharmacology & Toxicology, ²Division of Nephrology, Internal Medicine, Virginia Commonwealth University, School of Medicine, Richmond, VA. Recently, our lab has reported that Vav2, a member of the guanine nucleotide exchange factor (GEF) family contributes to the activation of NADPH oxidase (NOX) in a membrane lipid signaling platform in response to elevated levels of homocysteine (Hcys). However, it remains unknown whether Vav2-mediated NOX activation is able to trigger the NLRP3 inflammasome in podocytes and thereby lead to podocyte dysfunction and glomerular injury associated with hyperhomocysteinemia (hHcys). In our experiments, murine podocytes were pretreated with either a Rac-1 inhibitor, NSC23766 or Vav2 activator, uridine triphosphate (UTP) and then stimulated with Hcys for 24 hours. Confocal microscopic analysis showed that treatment with UTP increased the colocalization of inflammasome proteins NLRP3 with ASC or with caspase-1, suggesting inflammasome formation. However, pretreatment of podocytes with NSC23766 blocked Hcys-

induced inflammasome formation. Similar to Hcys, Vav2 activator, UTP increased caspase-1 activation and consequent IL-1 β production in podocytes. However, attenuated caspase-1 activation and lower IL-1 β levels were observed when podocytes were treated with NSC23766. Our results suggest that Vav2 is a key signaling molecule in mediating Hcys-induced podocyte inflammasome formation and activation and consequent podocyte dysfunction and glomerular injury.

NICOTINE PREVENTS CHEMOTHERAPY-INDUCED PERIPHERAL NEUROPATHY *IN VIVO*, AND FAILS TO STIMULATE THE GROWTH OF LUNG CANCER CELLS OR INTERFERE WITH THE EFFECTIVENESS OF CHEMOTHERAPY *IN VITRO*. S. L. Kyte¹, W. Toma¹, M. I. Damaj¹, X. Fang² & D.A. Gewirtz¹, ¹Dept. of Pharmacology & Toxicology and ²Dept. of Biochemistry & Molecular Biology, Virginia Commonwealth University, Richmond, VA 23298. Chemotherapy has played a significant role in the treatment and survival of cancer patients. However, its use can lead to long-term symptoms of drug toxicity, including chemotherapy-induced peripheral neuropathy (CIPN), a result of peripheral nerve fiber dysfunction or degeneration. Paclitaxel (Taxol), a taxane commonly used to treat breast, lung, and ovarian cancers, has been found to cause CIPN in 59 to 78% of patients. There is currently no effective preventative or therapeutic treatment for this side effect. Our studies revealed that the nicotinic acetylcholine receptor (nAChR) agonist, nicotine, is capable of reversing and preventing the development of paclitaxel-induced CIPN *in vivo*, and does not interfere with the cytotoxic properties of paclitaxel *in vitro*. The use of von Frey filaments revealed that nicotine dose-dependently reverses and prevents paclitaxel-induced mechanical allodynia. The *in vitro* studies showed that nicotine fails to significantly stimulate growth of A549, H460, Lewis lung carcinoma, or human explant lung cancer cells. Most importantly, paclitaxel-induced H460 growth inhibition was not significantly attenuated by nicotine. Moreover, nicotine failed to alter the sub-G1 DNA content of paclitaxel-treated A549 cells. These findings suggest that nAChRs may be promising drug targets for the prevention and treatment of CIPN.

DOWNREGULATION OF MYELIN GENE EXPRESSION IN THE ENTORHINAL CORTEX OF FINGOLIMOD TREATED MICE. Jessica L. Jurmain & Michael F. Miles, Dept. of Pharmacology & Toxicology, Virginia Commonwealth Univ., Richmond, Virginia, 23298. Studies in human and mouse models of alcoholism suggest a role for myelin in the development of alcohol use disorders (AUDs) and their associated pathologies. Myelin genes are downregulated in the frontal cortex of alcohol dependent patients and in mouse models of AUD. Studies in C57BL/6J and DBA/2J mice show correlations

between basal myelin gene expression and behavioral responses to acute ethanol exposure. Fingolimod (FTY720) is an FDA approved drug for the treatment of relapsing multiple sclerosis. Literature reports on FTY720 suggest a direct role for its receptor in demyelination and remyelination. The significant overlap of genes regulated in hippocampus of FTY720-treated immune-deficient mice with an ethanol responsive gene set from our laboratory included several myelin genes. This may suggest a common mechanism between the effects of FTY720 and chronic ethanol treatment on myelin gene expression that may be useful in elucidating a treatment for the myelin-related pathologies associated with ethanol use. Using qRT-PCR we examined the effects of oral FTY720 on myelin gene expression in ethanol-naïve mice. There were no significant changes in myelin gene expression in medial prefrontal cortex, caudate putamen, or nucleus accumbens. Two myelin genes, *Mbp* and *Plp*, were significantly downregulated in the entorhinal cortex of FTY720 treated mice, and this downregulation was strongly correlated with the downregulation of *NFκB* and *Tlr4* expression. This suggests that FTY720 may aid in elucidating a mechanism for demyelination associated with chronic ethanol use.

MISSENSE MUTATIONS IN GONADOTROPIN-RELEASING HORMONE RECEPTOR GENE IN PATIENTS WITH NORMOSMIC IDIOPATHIC HYPOGONADOTROPIC HYPOGONADISM. N. V. Zernov¹, M. Y. Skoblov^{1,2}, A. V. Baranova^{1,2,3,4} & K. Y. Boyarsky⁵, ¹Federal State Budgetary Institution Research Centre for Medical Genetics, Moscow, 115478, Russia, ²The Moscow Institute of Physics & Technology, Dolgoprudny, Moscow Region, 141700, Russia, ³Center for the Study of Chronic Metabolic Diseases, School of Systems Biology, College of Science, George Mason University, Manassas, VA, USA. ⁴Atlas Biomed Group, Moscow, 123317, Russia and ⁵Center of Human Reproduction "Genesis", Department of Obstetrics and Gynecology, State Pediatric Medical University, St.-Petersburg, 194100, Russia. Isolated hypogonadotropic hypogonadism (IHH) is a rare genetic disease occurring in about 1-10 cases per 100,000 births. In only 40% of these patients the sense of smell is unchanged. This normosmic IHH (nIHH) is due to a failure of gonadotropin-releasing hormone (GnRH) pulsatile secretion in hypothalamus or its action in pituitary. In nIHH patients, mutations are identified in genes *GnRH1*, *GnRHR*, *KISS1*, *KISS1R*, *TAC3* and *TAC3R*. We present our observations of two non-consanguineous Russian female patients diagnosed with nIHH. Prior to referral to fertility clinic, each patient underwent about 10 years of hormone replacement therapy. Treatment was successful in both patients who delivered phenotypically healthy offspring. Direct sequencing of *GnRHR* gene identified homozygous mutation c.416G>A in one patient and compound heterozygous c.

416G>A/c.806C>T in another patient. To our knowledge, this is the first observation of successful pregnancy of patients lacking GnRHR function.

HAS THE ERADICATION OF THE POLIO VIRUS CONTRIBUTED TO THE RISE IN SOLID TUMOR CASES IN RECENT YEARS? K. Gwilliam¹, A. Baranova^{1,2} & A. Biredinc^{1,2}, ¹Center for Study of Chronic Metabolic Disease, College of Science, George Mason University, Fairfax, VA 22030 and ²Betty and Guy Beatty Center for Integrated Research, INOVA Health System, Falls Church, VA 22042. A genetically altered version of the polio virus is being used in a Phase I study to treat glioblastoma multiforme (GBM) at the Preston Robert Tisch Brain Tumor Center at Duke University by Dr. Gromeier and his team. The genetically engineered poliovirus, PVS-RIPO, infects cells that have poliovirus receptors yet is disabled from replicating in normal, healthy cells. This raises the question of why and how polio virus can target glioblastoma cells and its evolutionary origins. This study uses publicly available databases, including the World Health Organization, to assess the administration of the inactivated and oral polio vaccine and the correlation incidence of polio and solid tumors reported on a global scale. Out of 69 countries studied, from five WHO regions, most displayed a decline in the incidence of poliomyelitis from 1988 onward. Many of the 69 countries also showed an increased number of cancer cases between 1983 to 2007. A multivariate analysis will be performed to determine if there is a correlation between the eradication of polio in the human population and the rise in solid tumors. Outcomes could reveal the polio virus' ability to regulate solid tumors as well as possible influences that vaccination may have had on this regulation. This information could assist in better administering the polio virus for the treatment of solid tumors, such as GBM, as well as provide valuable mechanistic and evolutionary insight into the co-evolution of the polio virus and humans.

EPIGENETICS IN THE ETIOLOGY OF CHRONIC DISEASES. K. Y. Jeong, A. Biredinc & A. Baranova, Dept. of Biology, George Mason University, Fairfax VA 22030 and Betty and Guy Beatty Center for Integrated Research, Inova Health System, Falls Church VA 22042. Several recent lines of research have shown that in addition to a genetically predisposed profile, certain epigenetic factors play an important role in both the development and progression of certain chronic diseases such as NAFLD. Particularly for diseases under the umbrella of Metabolic Syndrome, it has been demonstrated that epigenetic as well as genetic factors play a crucial role in both the presence and severity of these comorbidities. This article focused on incorporating the epigenetic regulations found with the etiological

pathways of chronic diseases. A systematic review was performed to assess the work done to date. The two main investigated areas inflicted by Metabolic Syndrome include the heart and the liver. Results found that the accumulation of visceral fat increases expression of lipase genes in the liver, leads to greater FFA mobilization and results in hyperlipidemia linked with Steatosis and cardiovascular diseases. Recruitment of cytokines released by adipocytes may be linked to increased DNA methylation of glucokinase gene and hypomethylation of hepatic cell cycle inhibitor *Cdkn1a*, which led to decreased and increased expression, respectively, and contribute to NAFLD. The subsequent review paper will aim to summarize and consolidate the relationship between the epigenetic and genetic contributions to chronic diseases under the umbrella of Metabolic Syndrome.

LIPID PROFILING IN CARDIOVASCULAR RISKS. S. Srivangipuram^{1,3}, T. Cui², J. Hamre², B. Veytsman², A. Birerdinc^{2,3} & A. Baranova^{1,2,3}, ¹College of Science, George Mason Univ., Fairfax VA 22030, ²School of Systems Biology, George Mason Univ., Manassas 20110, ³Center of Study of Chronic Metabolic Diseases, George Mason Univ., Falls Church., VA., Inova Health System, Falls Church., VA 22042. Different classes of High-density lipoproteins transport cholesterol in blood and their inverse correlation with cardiovascular disease (CVD) is well known. It is believed that HDL facilitates the removal of excess cholesterol from peripheral tissues and delivers it to the liver for excretion. Mass spectrometry was employed to identify 187 proteins and the amount of given protein detected in blood was collected from 101 patients who have varying levels of cardiovascular disease as measured by CT angiography. The HDL proteomics data was displayed as a weighted spectrum count. The statistical software “R” was used to perform descriptive and inferential statistics on the data. To see if the proteins are expressed differently in diseased samples, a preliminary analysis was conducted by comparing the median distance of the counts to the normal space center. Since the mild severity group was closer to the normal, these two groups were combined for distance based analysis. There was not a gravitation of the counts towards a specific severity. A Wilcoxon signed-rank test was used to test for significant differences in protein expression among the different severity groups ($\alpha=0.05$). There was no significant difference in protein expression between normal to moderate group, normal to severe, and moderate to severe. The results suggest for further analysis by separating the HDL binding proteins to see if they contribute to disease severity.

FIBRONECTIN FIBRIL-ASSOCIATED GROWTH FACTORS IN BREAST CANCER MICROENVIRONMENTS. Pascal Shukuru & Lynne Elmore, Dept. of Pathology, Virginia Commonwealth University,

Richmond VA 23298. Fibronectin (FN) is expressed in many breast cancers (BC) and implicated in tumor progression. FN forms fibrils, which can bind >40 soluble growth factors, many with pro-oncogenic properties. Data indicate that breast adipose-derived mesenchymal stem cells (bMSCs) promote BC cell growth and invasion as well as the development of a FN-rich extracellular matrix. These resident stem cells also express numerous FN binding growth factors, including TGF- β 1, and assembly FN fibrils, which bind latent TGF- β 1 binding protein 1 (LTBP1), an activator of TGF- β 1. These experimental data prompted us to investigate whether TGF- β 1 and/or LTBP1 co-localize with FN fibrils in clinical specimens of BC. Tissue microarrays were stained with Masson's Trichrome to identify fibrosis. Immunohistochemistry was performed to assess protein expression levels and localization of TGF- β 1 and LTBP-1. Many BC specimens exhibited abundant FN-rich fibrotic stroma. TGF- β 1 co-localized with FN fibrils in the extracellular matrix of many BC tissues, while LTBP1 was expressed at variable levels in BC and stromal cells. These data provide a foundation for testing whether co-localization of FN binding growth factors (or mediator of these growth factors) and FN fibrils associate with severity of disease.

ABUSE-RELATED EFFECTS OF GABA_A RECEPTOR POSITIVE ALLOSTERIC MODULATORS IN AN ASSAY OF INTRACRANIAL SELF-STIMULATION IN RATS. K.L. Schwientek¹, G. Li², M. M. Poe², J. M. Cook², M.L. Banks¹ & S.S. Negus¹, ¹Dept. of Pharmacology & Toxicology, Virginia Commonwealth University, Richmond, VA, 23298 and ²Dept. of Chemistry & Biochemistry, University of Wisconsin-Milwaukee, Milwaukee, WI, 53211. GABA_A receptor positive allosteric modulators (GABA_A PAMs) are used clinically but also have abuse liability. Novel GABA_A PAMs have been developed that vary in efficacy at, and selectively for, GABA_A receptor subtypes that contain α 1, α 2 or α 3 subunits. Intracranial self-stimulation (ICSS) is one preclinical procedure that has been used to evaluate abuse potential of drugs. This study compared effects on ICSS produced by diazepam (high-efficacy and relatively non-selective), zolpidem (high-efficacy and selective for GABA_A receptors containing an α 1 subunit), and the compounds JY-XHe-053, XHe-II-053 and HZ-166 (intermediate-efficacy with putative selectivity for GABA_A receptors that contain α 2/ α 3 subunits). Adult, male Sprague-Dawley rats (n=17) were trained in an ICSS procedure. Diazepam (0.1-10 mg/kg) and zolpidem (0.032-3.2 mg/kg) produced transient abuse-related effects at low doses. JY-XHe-053 (3.2-32 mg/kg) and HZ-166 (3.2-32 mg/kg) produced significant but weaker and less reliable effects, and XHe-II-053 (3.2-32 mg/kg) had no effect. These results are consistent with other evidence for abuse potential of diazepam and zolpidem and also suggest that

high efficacy and/or selectivity at $\alpha 1$ GABA_A receptor subtypes contributes to abuse-related effects of GABA_A PAMs. Supported by NIH grants R01-NS070715, R01-MH096463, and VCU School of Medicine.

ANTI-INFLAMMATORY DIDOX AS A TREATMENT IN A NEW PROGRESSIVE MODEL OF EAE. D. J. Adkins¹, K. C. Clark², M. Joslyn², G. H. DeVries² & J. L. Dupree², ¹Thomas Nelson Community College, Hampton VA, 23666 and ²Dept. of Anatomy & Neurobiology, Virginia Commonwealth University, 23284. Multiple sclerosis is an autoimmune inflammatory disease that presents with motor, sensory and cognitive impairment. In this study we used a mouse model of MS, known as experimental autoimmune encephalomyelitis (EAE), to investigate the efficacy of a novel anti-inflammatory drug as a potential treatment for progressive MS. We induced a progressive form of EAE in four mice which were monitored daily as they developed the disease. At peak disease stage, two animals were administered the novel drug, known as didox, for five consecutive days. Based on previous observations from our lab using a chronic model of EAE, we hypothesized that 1) the mice at peak disease would lose specific axonal domains known as axon initial segments (AIS) in the brain and nodes of Ranvier in the spinal cord; 2) that microglia, the resident immune cells of the central nervous system, would make contact with AIS and nodes of Ranvier; and 3) that the didox treated mice would exhibit recovery of their AIS and nodes of Ranvier. Consistent with our hypothesis, we found that both AIS and nodes of Ranvier were reduced and microglia contacted ~20% of all AIS and all nodes of Ranvier. Lastly, we found a partial recovery of AIS and nodes of Ranvier in the didox treated mice. We conclude that in this progressive EAE model, didox treatment may facilitate the reclustering of proteins essential for AIS and node of Ranvier function.

NETWORK ANALYSIS OF CHRONIC ETHANOL RESPONSIVE GENE EXPRESSION IN PREFRONTAL CORTEX REVEALS CONSERVED CHANGES ACROSS MOUSE AND MACAQUE. M.L. Smith¹, J.W. Bogenpohl¹, C. Helms², M.F. Lopez³, K.A. Grant², H.C. Becker³ and M.F. Miles¹, ¹Dept. of Pharmacology & Toxicology, Virginia Commonwealth Univ., Richmond, VA 23298, ²Dept. of Behavioral Neuroscience, Oregon Health & Science Univ., Portland, OR 97239, and ³Dept. of Psychiatry, Medical University of South Carolina, Charleston, SC 29425. Alcohol use disorder (AUD) is a significant public health problem. Characteristic features include craving, withdrawal, and increased consumption. Here we use two animal models explore gene expression responses in the prefrontal cortex (PFC). Ethanol's effect on PFC is of interest due to its role in executive function. C57BL/6J mice were exposed to ethanol by chronic

intermittent ethanol (CIE) with 2 bottle choice drinking. Rhesus macaques were exposed to ethanol using schedule induced polydipsia (SIP). Gene expression was measured using Affymetrix microarrays, and expression data was analyzed using Weighted Gene Correlated Network Analysis (WGCNA). With WGCNA, groups of genes showing significantly correlated expression in both mice and monkeys were identified. Gene Ontology analysis revealed that modules represented known biological processes including neurotransmission, myelination, mitochondrial respiration, and regulation of gene expression. These results indicate a conserved gene expression response to chronic ethanol exposure in mice and monkeys. These consensus modules, therefore, reveal biological themes in the conserved response to chronic ethanol exposure across species, and may represent therapeutic targets to modulate the behavioral features of AUD such as escalating ethanol consumption.

PACLITAXEL-INDUCED NEUROPATHY AND MECHANICAL ALLODYNIA DO NOT CORRELATE WITH BEHAVIORAL DEPRESSION IN RATS. Luke P. Legakis & S. Stevens Negus, Dept. of Pharmacology & Toxicology, Virginia Commonwealth University, Richmond, VA. Paclitaxel is a cancer chemotherapy drug with adverse effects that include chemotherapy-induced peripheral neuropathy (CIPN), neuropathic pain, and depression of mood and behavior. These adverse effects can limit the clinical use of paclitaxel and reduce patient well-being for decades. Preclinical research on expression and treatment of paclitaxel-induced neuropathic pain has relied almost exclusively on hypersensitive reflex-withdrawal responses to mechanical or thermal stimuli as the primary measure of "pain." It is unknown if paclitaxel can also produce signs of pain-depressed behavior in animals. The objective of this study was to evaluate paclitaxel effects on rates of positively reinforced operant responding in rats as a measure of functional impairment and behavioral depression. The specific aim was to test the hypothesis that regimens of paclitaxel treatment sufficient to produce neuropathy and mechanical allodynia would also depress rates of positively reinforced operant responding in assays of intracranial self-stimulation (ICSS) and food-maintained responding. Paclitaxel decreased rates of both ICSS and food-maintained responding in some rats, but the magnitude of depression was not statistically significant in analysis of group data. Moreover, in analysis of individual data, the magnitude of depression in rates of operant responding did not correlate with either IENF loss or decreases in mechanical sensitivity threshold. These results suggest that neuropathy and mechanical allodynia do not cause behavioral depression and may have different mechanisms than behavioral depression.

ETHANOL REGULATION OF NDRG1 AND THE EFFECTS OF PFC MODULATION OF NDRG1 ON DRINKING BEHAVIOR. G. M. Harris, A. D. van der Vaart, S. O. Park & M. F. Miles, Dept. of Pharmacology & Toxicology, Virginia Commonwealth University, Richmond, VA 23298. Multiple molecular, pathological and neuroimaging studies show perturbation of myelin or myelin gene expression in alcoholics. Prior microarray studies in our laboratory found down-regulation of myelin-related genes in prefrontal cortex (PFC) of human alcoholic post mortem tissue as well as regulation of myelin genes by acute ethanol exposure in mouse PFC. A measure of acute sensitivity to ethanol that is frequently used in animal behavioral studies is the Loss of Righting Reflex (LORR) duration. Our laboratory has implicated N-myc down-regulated gene 1 (*NdrG1*) as a potential candidate gene that modulates ethanol-induced changes in myelin-related gene expression and acute sensitivity to ethanol. Analysis of PFC expression data found that *NdrG1* expression was positively correlated with ethanol intake across the BXD panel of mice and demonstrated that the basal levels of *NdrG1* mRNA expression in the PFC across seven different strains of mice was inversely correlated with LORR duration time. PFC-specific knockdown of *NdrG1* mRNA by stereotactic injection of lentivirus expressing *NdrG1*-shRNA in B6 mice, caused increased ethanol LORR duration and decreased preference for ethanol. While CNS *NdrG1* is thought to be expressed mainly in oligodendrocytes, we have observed it co-localized within neurons in the PFC. A detailed characterization of *NdrG1* regulation at the mRNA and protein expression level following acute or chronic ethanol is currently underway. Viral methods are also being developed to up-regulate *NdrG1* expression in oligodendrocytes and pyramidal neurons within the PFC.

EFFECTS OF CHRONIC AMPHETAMINE ON BEHAVIORAL AND NEUROCHEMICAL EFFECTS OF COCAINE IN RATS. Amy R. Johnson & S. Stevens Negus, Dept. of Pharmacology & Toxicology, Virginia Commonwealth Univ., Richmond, VA. Amphetamine maintenance decreases cocaine use in preclinical studies and clinical trials. The mechanisms underlying the anti-cocaine effects of amphetamine are not well understood. This study evaluated abuse-related effects of cocaine on intracranial self-stimulation (ICSS) and on nucleus accumbens dopamine and serotonin (NAc DA and 5HT) levels. We hypothesized that amphetamine maintenance would decrease both cocaine-induced ICSS facilitation and enhancement of NAc DA. Male Sprague-Dawley rats were used for all studies. For ICSS, electrodes were implanted in the medial forebrain bundle, and responding on a lever produced pulses of electrical brain stimulation in a frequency-rate ICSS procedure. Effects of cumulative cocaine doses (1-10 mg/kg IP) were determined before and after 7-day treatment with saline, 0.1 or

0.32 mg/kg/hr amphetamine delivered by a subcutaneous osmotic minipump. For microdialysis, rats were implanted with cannulae targeting the NAc, and dialysates were analyzed for concentrations of DA and 5HT before and after 10 mg/kg IP cocaine. Cocaine facilitated ICSS and increased NAc levels of both DA and 5HT. Amphetamine maintenance facilitated ICSS throughout treatment and eliminated cocaine-induced ICSS facilitation. Amphetamine maintenance also increased basal DA concentration and eliminated cocaine-stimulated increases in NAc DA without affecting basal 5HT concentration. These results suggest that amphetamine maintenance decreases abuse-related behavioral effects of cocaine by decreasing cocaine-induced increases in mesolimbic DA. Supported by R01DA026946.

IMMUNOLOGICAL LANDSCAPE OF NSCLC: OPPORTUNITIES FOR INTERVENTION? Se W. Jeong & Timothy N.J. Bullock, Dept. of Pathology, University of Virginia, Charlottesville, VA 22908. Lung cancer is the leading cause of annual cancer related mortality in the United States. More than 150,000 people in the US will die from lung cancer this year which will lead to roughly as many deaths as breast, prostate, colon, and pancreatic cancers combined. Lung cancer is also of global concern as tobacco use in developing countries has risen significantly. Stage II and III non-small cell lung cancer (NSCLC) patients often have a 5-year disease free survival of less than 50% even after surgical resection and post-operative chemotherapy and a dismal 5% overall survival for patients with metastatic disease. Even with the advent of PD-1/PDL-1 checkpoint blockade immunotherapies, only about 20% of patients are responsive to the treatment. This limited scope of success may be due to our lack of understanding the immune landscape of NSCLCs. Therefore, we set out to characterize the immune composition from surgical lung resections via flow cytometry. We found that NSCLCs have a diverse set of checkpoint inhibitor and co-stimulatory molecules expressed by T cells, including PD-1, TIM-3, and TIGIT. Interestingly, even though PD-1 was elevated compared to a normal donor, the expression level was lower than either breast or melanoma tumor samples. In addition, regulatory T cell populations have a profound presence in these lung tumors. These findings suggest potential molecules as targets of interest for immunotherapy but also the importance of recognizing the diversity of immune populations that exist within a tumor.

EFFECTS OF TRAUMATIC BRAIN INJURY ON OXYCODONE REINSTATEMENT AND PHYSICAL DEPENDENCE. Neil B. Varshneya & Katherine L. Nicholson, Dept. of Pharmacology & Toxicology, Virginia Commonwealth University, Richmond, VA 23298. Epidemiological data indicate that patients who experience a traumatic brain injury (TBI) have an elevated risk of developing a substance use

disorder (SUD), however the underlying neurobiological connections remain unclear. We investigated the effects of TBI on the abuse-related effects of oxycodone in preclinical models. Our evaluation utilized a lateral fluid percussion injury of moderate severity in adult male Sprague-Dawley rats. In the first aim, we tested the hypothesis that moderate TBI increases the risk for relapse to an opioid use disorder as measured by reinstatement of lever-pressing behavior following extinction in an intravenous oxycodone self-administration procedure. In the second aim, we tested the hypothesis that moderate TBI increases physiological dependence to oxycodone as measured by decreases in food-reinforced lever-pressing behavior and increases in other withdrawal behaviors in both precipitated withdrawal and spontaneous withdrawal. In reinstatement tests, non-injured subjects reinstated responding under oxycodone-associated cue- and oxycodone prime-induced conditions, however, brain-injured subjects did not reinstate lever-pressing behavior under any conditions. In dependence tests, brain-injured subjects showed no significant differences from non-injured subjects in mean withdrawal scores or food-reinforced lever-pressing behavior. Overall, these data suggest that brain-injured patients with no significant pre-morbid history of opioid abuse have lesser risk of relapse to opioid use disorders. Supported by Department of Defense, W81XWH-11-1-0374.

ETHANOL REVERSAL OF OPIOID TOLERANCE IN MICE.
Joanna C. Jacob & William L. Dewey, Dept. of Pharmacology & Toxicology, Virginia Commonwealth University, Richmond, VA 23298. Prescription opioids are generously prescribed due to their highly effective antinociceptive properties, however their use is limited by a high abuse potential due to euphoric effects. Chronic opioid use, whether for clinical or recreational purposes, is often coupled with the use of other substances, such as ethanol. Dangerous drug interactions are known to occur with opioids and ethanol, yet the mechanisms are not understood. This led to the investigation and hypothesis that ethanol reverses tolerance to at least some opioid effects, such as analgesia and respiratory depression, ultimately leading to overdose and death. We found a significant and dose-dependent reversal of morphine-induced analgesic tolerance by ethanol, as measured by warm-water tail withdrawal in Swiss Webster mice. Similar studies were carried out for oxycodone and hydrocodone, where analgesic tolerance was developed via chronic s.c. injections of an ED₈₀ dose, and then altered by a single i.p. injection of 1g/kg ethanol. The ED₅₀ for oxycodone was significantly shifted from 0.9 mg/kg (0.72 – 1.12) to 1.70 mg/kg (1.42 – 2.03) after repeated administration and returned to 1.02 mg/kg (0.77-1.37) after 1 g/kg ethanol administration. Similarly, the ED₅₀ for hydrocodone was shifted from 3.92 mg/kg (3.26 – 4.71) to 9.01 mg/kg (6.44 – 12.62) and returned to 4.73 mg/kg (3.51 – 6.38) after 1 g/kg

ethanol administration. Together these data support the hypothesis that ethanol reverses analgesic tolerance to opioid drugs in addition to morphine, and could be working through the same mechanisms.

PROLIFERATIVE RECOVERY AND REVERSIBILITY OF THERAPY-INDUCED SENESENCE IN NON-SMALL CELL LUNG CANCER. Tareq Saleh & David A. Gewirtz, Virginia Commonwealth Univ., Dept. of Pharmacology & Toxicology, Richmond, VA. Lung cancer is the leading cause of cancer-related death in both men and women in the United States. Most lung cancer cases are diagnosed in advanced, inoperable stages and are treated with chemoradiation; while chemoradiation is effective in suppressing tumor progression, recurrence following treatment is not infrequent. The involvement of autophagy, senescence and apoptosis in the actions of etoposide, one of the primary drugs utilized in the treatment of non-small cell lung cancer (NSCLC), was studied in H460 NSCLC cells. Exposure to etoposide resulted in growth arrest accompanied by the induction of senescence, but minimal apoptosis. Growth arrest was transient in that proliferative recovery was evident by day 7 post exposure. Quantification of senescence over time based on C₁₂FDG staining and flow cytometry demonstrated that the reversal of growth arrest coincided with a decline in the extent of senescence. To more precisely define the source of the recovered cells, senescent and non-senescent but growth arrested cells were separated by flow cytometry based on their relative β -galactosidase expression and replated. Both cell populations demonstrated the ability to re-emerge from the growth-arrested state and recover proliferative capacity. These observations suggest that senescence is ultimately a transient process in that at least a subpopulation of tumor cells can and will recover proliferative capacity. We propose that the reversibility of therapy-induced senescence (TIS) might be developed as a model for studies of tumor dormancy and disease recurrence.

MOLECULAR MODELLING AND VIRTUAL SCREENING OF *PLASMODIUM FALCIPARUM* GLYCOGEN SYNTHASE KINASE 3 (*pf*GSK-3) FOR ATP NON-COMPETITIVE INHIBITORS. S. Obeng, S. A. Zaidi, P. D. Mosier & Y. Zhang, Dept. of Medicinal Chemistry, Virginia Commonwealth University, Richmond VA 23298-0540. The purpose of this study was to screen for new lead compounds that are ATP non-competitive *pf*GSK-3 selective inhibitors to treat malaria. Since there is no crystal structure of *pf*GSK-3 available, a blast search of the PDB database was carried out. The human glycogen synthase kinase 3 β (*h*GSK-3 β) (PDB: 4ACC) was identified as the enzyme with the highest homology to *pf*GSK-3 with a sequence identity and similarity of 42.9% and 60.4% respectively. Using *h*GSK-3 β as the template, 100 models of *pf*GSK-3 were constructed using MOD9.14.

The best model was selected by docking a known *pf*GSK-3 ATP non-competitive inhibitor (manzamine) into a binding pocket comprising residues Arg 96, Arg 180, Lys 205, and Tyr 216. The best model obtained was used to carry out a virtual screening of the NCI and ZINC-sigma aldrich libraries using UNITY. The hits obtained were docked into the *pf*GSK-3 homology model using GOLD52 and the scoring function CHEMPLP. Three compounds with CHEMPLP scores of 111.07, 97.81, and 95.47 were identified as lead compounds.

NOVEL PHOTOAFFINITY PROBES FOR α -N-TERMINAL WRITERS. B. D. Mackie¹, S. L. Richardson² & R. Huang¹, ¹Dept. of Medicinal Chemistry and ²Dept. of Chemistry, Virginia Commonwealth University, Richmond VA 23219. Protein α -N-terminus undergoes a variety of modifications including methylation, acetylation, myristoylation, and palmitoylation. It has been hypothesized that there is dynamic interplay among those modifications, such as methylation and acetylation. Methylation has recently demonstrated its important role in regulating protein-DNA interactions, mitotic division and DNA damage repair. Acetylation is involved in protein degradation, localization, and complex formation. To explore these dynamic modifications, we developed photoaffinity probes to profile enzymes that are responsible for α -N-terminal modifications. We have successfully synthesized a photoaffinity probe which contains three main components: a recognition element, a photocrosslinker, and a fluorescent tag. The recognition element is crucial for selectivity, the photocrosslinker is needed to covalently bond the probe to the target and the tag is necessary for fluorescence imaging. The photoaffinity probe's recognition element was derived from the N-terminus of Retinoblastomal 1 (RB1), a substrate of N-terminal methyltransferase 1 (NTMT1). Our results suggest that our probe exhibits in a dose dependent, time dependent and competitive manner. Photoaffinity labeling was competitively inhibited when NTMT1 was incubated with the probe and varying concentrations of RB1-10. The RB1 probe also selectively labeled NTMT1 when NTMT1 was spiked into a nuclear extract cell line, verifying specificity of the probe for NTMT1. Lastly, the probe was enzymatically methylated by NTMT1, giving further validation that labeling is driven by recognition.

IMPROVING THE SIDE EFFECT PROFILE OF ANTICOAGULANTS USING THROMBIN ALLOSTERISM. D. K. Afosah^{1,2}, S. Verespy^{2,3}, R. Karuturi^{1,2}, R.S. Boothello² & U. R. Desai^{1,2}, ¹Dept. of Medicinal Chemistry and ²Institute for Structural Biology, Drug Discovery and Development, Virginia Commonwealth University, Richmond VA 23219, and ³Dept. of Chemistry, Virginia Commonwealth University, Richmond VA 23284. Thrombin is a serine protease that occupies a central position in the coagulation cascade. Its

key feature is the conversion of fibrinogen to fibrin, a key component of blood clots. Currently, all thrombin inhibitors on the market inhibit the protease fully resulting in increased risk of bleeding with their use. We reasoned that it should be possible to develop partial inhibitors of thrombin that allow reasonable proteolytic activity even at saturating concentrations of the inhibitor. Based on our earlier work with sulfated benzofuran dimers, which showed 75% inhibition at saturation concentrations, we designed advanced analogs using computational virtual screening. These analogs were synthesized and their biological profile studied using biochemical assays. The results show that a distinct group of analogs display inhibition efficacies of ~50-60% at saturation in chromogenic substrate assay, whereas others exhibit efficacies of $\geq 80\%$. Similar results were observed in the fibrinogen assay suggesting that submaximal inhibition is maintained with thrombin's *in vivo* substrate. The results indicate that thrombin's high plasticity can be exploited to realize a clinically relevant homeostatic inhibitor that resolves bleeding risk.

INTERPLAY BETWEEN ARTEMIS AND TDP1 IN SENSITIVITY TO RADIOMIMETIC AGENTS. A. Kawale, K. Akopiants & L. F. Povirk, Dept. of Pharmacology & Toxicology, Virginia Commonwealth University, Richmond, VA 23298. DNA double-strand breaks (DSBs) containing unligatable termini are potent cytotoxic lesions leading to growth arrest or cell death. Artemis, which is associated with the Non-Homologous End Joining (NHEJ) pathway, is the major end processing nuclease that resolves unligatable termini, especially the 3' blocks, by nucleolytic trimming. Tyrosyl-DNA Phosphodiesterase 1 (TDP1) is an enzyme which is biochemically competent in 3'-phosphoglycolate processing. The purpose of this study is to investigate if TDP1 is an end-processing enzyme involved in the NHEJ pathway. A cell line with combined deficiency in Artemis and TDP1 was generated by infecting Artemis^{-/-} single mutants with a lentivirus expressing a TDP1 shRNA. Positive clones were screened for maximum TDP1 knockdown which was found to be around 14X. Clonogenic survival assays carried out on shTDP1 & Artemis^{-/-} single mutants and the Artemis^{-/-}.shTDP1 double mutants showed similar sensitivity to NCS and Calicheamicin. Thus, surprisingly, these experiments suggest that TDP1 functions are epistatic with Artemis in the NHEJ pathway for repair of DNA double-strand breaks.

HYBRID MOLECULES AS TOOLS TO STUDY CLASSICAL 5-HT_{2A} RECEPTOR LIGANDS. U. H. Shah¹, S. A. Gaitonde¹, J. L. Moreno², J. Gonzalez-Maeso², M. Dukat¹ & R. A. Glennon¹, ¹Dept. of Medicinal Chemistry, and ²Dept. of Physiology & Biophysics, Virginia Commonwealth University, Richmond VA 23298. Serotonin-2A (5-HT_{2A}) receptor antagonists have therapeutic applications in

schizophrenia and belong to diverse chemical classes. Current pharmacophore models for 5-HT_{2A} receptor antagonists suggest that these agents might have multiple binding modes. Reported pharmacophores include *two* aromatic/hydrophobic regions and a protonated amine. Hybrid molecules (Ket/Ris and Ris/Ket) of two known 5-HT_{2A} antagonists, ketanserin (Ket) and risperidone (Ris), were synthesized by our laboratory to study their binding modes. Binding data showed that Ket/Ris ($K_i = 0.96$ nM) binds with 19-, 5- and 13- fold higher affinity than Ket, Ris and Ris/Ket, respectively. The high affinities of Ris and Ket/Ris can be attributed to an additional bifurcated interaction as shown in homology models of 5-HT_{2A} receptors, which is not possible with the benzoyl ring in Ket and Ris/Ket. We synthesized deconstructed analogs of Ris and Ket to determine which portions contribute to 5-HT_{2A} receptor affinity and antagonism. 4-(4-Fluorobenzoyl) piperidine and FBIP, deconstructed analogs of Ket and Ris, respectively, were found to be 5-HT_{2A} receptor antagonists. Therefore, we have successfully identified a new pharmacophore for 5-HT_{2A} receptor antagonists that consists of *one* aromatic region, hydrogen bond acceptors, and a basic protonated amine. FBIM (K_i ca 12 nM), a deconstructed analog of Ris ($K_i = 5$ nM), was identified as a new high-affinity 5-HT_{2A} receptor antagonist. Published 5-HT_{2A} receptor antagonist models will now need to be completely revised.

THE TALE OF THREE TESTS: PARADOXICAL MODULATION OF OXYCODONE-INDUCED ANTINOCICEPTION BY LORCASERIN. Kumiko M. Lippold & William L. Dewey, Dept. of Pharmacology & Toxicology, Virginia Commonwealth Univ., Richmond, VA, 23298. Prescription opioids are important therapeutic agents for the treatment of pain that with chronic use, produce tolerance, dependence, and in many cases, addiction. There is a need to develop new approaches for minimizing the risk of opioid abuse and reducing overdose-related deaths. The underlying neurobiological mechanisms of these conditions suggest a modulatory role of the serotonergic system in the pharmacological effects of opiates. Lorcaserin is a selective agonist, at the serotonergic 5-HT_{2C} receptor, approved by the FDA for the treatment of obesity but recently investigated as a potential treatment for drug abuse and dependence. Preclinical studies have demonstrated the efficacy of lorcaserin to reduce the abuse-related effects and dependence behaviors of opiates. We evaluated lorcaserin in the acute effects of oxycodone using tests of nociception and observed paradoxical modulation of the antinociceptive effects of oxycodone, in a divergent manner, in models of spinally-mediated and supraspinally-mediated thermal nociception. Lorcaserin potentiated the antinociception produced by oxycodone in the tail immersion test (a spinally-mediated reflex) and in the hot plate assay (a test of supraspinally-mediated pain), there was an attenuation

of the antinociceptive effects of oxycodone. Interestingly, lorcaserin also suppressed stretching activity in mice that received an intraperitoneal injection of acetic acid but when administered prior to oxycodone, no significant effects were observed. These data suggest a contrasting role of the 5-HT_{2C} receptor in the effects elicited by oxycodone in the brain, spinal cord, and viscera.