samples were taken. LaMotte test kits were used for each test, and the nitrate and phosphate levels were measured with a colorimeter. The storm water management ponds exhibited normal ranges in chemistry values, with high nutrient levels in the Best Buy, Kohl's and Upper Target ponds still not exceeding EPA maximum dose levels. These ponds also exhibit higher alkalinity and water hardness levels and are constructed with culverts of concrete that is weathering, which could contribute to the elevations. Preparations are being made to monitor rainwater and surface runoff prior to entering the stormwater management ponds.

Medical Science

A BIOMARKER PANEL FOR NON-ALCOHOLIC STEATOHEPATITIS (NASH) AND NASH-RELATED FIBROSIS. Zobair M. Younossi[1,2,3], Sandra J. Page[2,3], Nila Rafiq[1,2], Aybike Birerdinc[2,3], Maria Stepanova[1,2], Noreen Hossain[2], Arian Afendy[1,2], Zahra Younoszai[1,2], Zachary Goodman[4] & Ancha Baranova[1,2,3], [1]Center for Liver Diseases, Inova Fairfax Hospital, [2]Betty and Guy Beatty Center for Integrated Research, Inova Health System, Falls Church, VA, [3]Center for the Study of Genomics in Liver Diseases, Molecular and Microbiology Department, George Mason University, Fairfax, VA, [4]Armed Forces Institutes of Pathology, Washington, DC. Non-alcoholic Fatty Liver Disease (NAFLD) is one of the most prevalent forms of chronic liver disease worldwide. Patients with NASH and NASH-related fibrosis, both intermediate stages of NAFLD, are at increased risk for progressive liver disease. Liver biopsy is used to diagnose these stages but has inherent risks; thus, a non-invasive alternative is greatly needed. This study examines the performance of a new, serum-based biomarker panel for NASH and NASH-related fibrosis. Serum from patients with biopsy-proven NAFLD was assayed for markers associated with the pathology of NASH and fibrosis. Regression models predictive of NASH, NASH-related fibrosis and NASH-related advanced fibrosis were then designed and cross-validated. The resulting models had AUC values > 80%, indicating high sensitivity and specificity. Together, these models formed a biomarker panel for NASH and NASH-related fibrosis that had good performance and was easy to use. Further testing on larger populations is warranted.

EXPRESSION OF CYTOKINES AND GASTRIC PEPTIDES IN MORBIDLY OBESE PATIENTS WITH NON-ALCOHOLIC FATTY LIVER DISEASE. Amanda C. Zirzow[1,2], Michael Estep[2], Noreen Hossain[2], Zachary Goodman[2], Hazem Elariny[2], Vikas Chandhoke[1], Ancha Baranova[1,2] & Zobair M. Younossi[2], [1]George Mason University, Fairfax VA 22030 and [2]Betty and Guy Beatty Center for Integrated Research, Inova Health Systems, Falls Church. Non-alcoholic fatty liver disease (NAFLD) describes the spectrum of conditions ranging from simple steatosis, the accumulation of excessive intercellular fat in hepatocytes, to non-alcoholic steatohepatitis (NASH), which is marked by necroinflammation and hepatic fibrosis. Although simple steatosis is relatively benign, 10 to 15 percent of the population will progress to NASH. Currently, the only way to diagnose NASH or to assess the stage of fibrosis is by obtaining a liver biopsy, which is invasive, expensive, and associated with

potential risks. This lack of diagnostics is intolerable since NAFLD occurs in an estimated 25 to 30 percent of the US general population, and NASH is reported in 2 to 3 percent of the population. NAFLD is closely associated with obesity, a chronic inflammatory state. This study investigates the role of the gastric appetite regulating peptides Ghrelin and Obestatin as well as several inflammatory cytokines by measuring their expression in the context of NAFLD. Data generated by this work could have direct relevance to patient diagnosis and screening as well as advance scientific understanding pertaining to the complex regulation of appetite stimulation and suppression in the context of obesity related disease.

ALPHA-MELANIN STIMULATING HORMONE (α-MSH) AND MELANIN CONCENTRATING HORMONE (MCH) EXPESSION IN OBESITY AND OBESITY RELATED DISEASES. Massih Abawi[1,2], Michael Estep[2], Vikas Chandhoke[1], Zobair M. Younossi[2], & Ancha Baranova[1,2]. [1]George Mason University, Fairfax VA 22030 and [2]Betty and Guy Beatty Center for Integrated Research, Inova Health Systems, Falls Church. In humans, melanin is produced in melanocytes and a few other specialized cells of the body. Our lab has been the first to demonstrate that melanin biosynthesis pathway is functional in adipose tissue of morbidly obese subjects. Melanin biosynthesis is regulated by melanogenic peptides αMSH and MCH. The aim of this study was to assess circulatory levels of αMSH and MCH in morbidly obese patients with obesity related diseases. Clinical data and fasting serum samples were collected from 39 morbidly obese NAFLD patients at the time of the liver biopsy. All liver biopsies were interpreted by a single hepatopathologist and assessed for liver disease. Fasting serum was assayed for αMSH and MCH concentrations which were determined by sandwich ELISA. Cytokine concentrations were obtained by BioPlex Multi-plex assay. Circulating levels of MCH and αMSH displayed strong positive correlation ($r=0.76$, $p<0.001$). Concentrations of αMSH showed small but statistically significant positive correlations with IL-6 ($r=0.36$, $p<0.05$), and Kupfer cell inflammation ($r=0.385$, $p<0.05$). Circulating concentrations of MCH also showed positive correlations with IL-6 ($r=0.32$, $p<0.05$), and Kupfer cell inflammation ($r=0.35$, $p<0.05$). Circulating levels of MCH and αMSH significantly correlate with markers of inflammation and may participate in the pathogenesis of NAFLD.

A CALCINEURIN-DEPENDENT LOSS AND AN OVERGROWTH OF DENDRITIC SPINES AFTER TRAUMATIC BRAIN INJURY IN RAT. John N. Campbell, Brian Low, David R. Register, & Severn B. Churn, Dept. of Neurology, Virginia Commonwealth University, Richmond VA 23298. Traumatic brain injury (TBI) can cause cognitive dysfunction in the absence of cell death, likely due in part to changes in neuronal connectivity. Dendritic spines form most of the excitatory synapses in the brain, and thus are one measure of neuronal connectivity. A loss of dendritic spines has been reported after TBI in human tissue samples, but this effect and its underlying mechanisms have not before been examined in an experimental model. In the present study, a modified Golgi-Cox technique was used to investigate the effect of TBI on dendritic spines at 1 h, 24 h, and 1 wk after lateral TBI in the adult rat. Principal cell dendrites were sampled for spine density in layer II/III neocortex,

hippocampal CA1 and CA3, and dentate gyrus. By 24 h post-TBI, the density of pedunculated (thin or mushroom-shaped) spines had decreased by 30% in ipsilateral layer II/III neocortex ($p<0.05$; n=19), by 29% in ipsilateral CA1 ($p<0.001$; n=18), and by 23% in contralateral CA1 ($p<0.01$; n=12). Strikingly, this loss of spine density was prevented by a single, 1 h post-TBI administration of the calcineurin inhibitor, FK506. By 1 wk post-TBI in untreated subjects, dendritic spine density returned to control levels in some brain regions but increased above control levels in other regions (ipsi CA1; +52%, $p<0.001$, n=14; contra CA1; +43% , $p<0.001$, n=13; ipsi CA3; +34%, $p<0.01$, n=14; contra CA3; +25%, $p>0.05$, n=13). These data imply significant, bilateral changes in the synaptic circuitry of the laterally-injured brain. Research supported by Commonwealth Neurotrauma Initiative grant 07-302E to S.B.C.

HERMITAMIDE B: DISCOVERY OF A MARINE NATURAL PRODUCT SODIUM CHANNEL BLOCKER. Eliseu O. De Oliveira[1], Kristin M. Graf[1], Kan Wang[1], Sivanesan Dakshanamurthy[1], Milton L. Brown[1], Manoj K. Patel[2], & Mikell Paige[1], [1]Drug Discovery Program, Georgetown University, Washington DC 20057 and [2]Dept. of Anesthesiology, University of Virginia, Charlottesville VA 22908. Marine natural products have historically been an important source of new drugs. The cyanobacterium *Lyngbya majuscula* present in tropical and sub-tropical waters produce a range of cytotoxic secondary metabolites. The lipopeptide hermitamide B was isolated from *L. majuscula* collected from deep-water coral reefs at Hermit Island Village, in Papua New Guinea. Because of its structural similarity with the jamaicamides, a family of sodium channel blockers isolated from cyanobacterium, we hypothesized that hermitamide B would also behave as a sodium channel blocker. We were delighted to find in our initial *in vitro* screen that hermitamide B displaces [^{3}H]-BTX from sodium channels in a comparable manner with phenytoin (i.e. ~20%, at 10 μM), a clinically used sodium channel blocker. Subsequent electrophysiology experiments showed that hermitamide B significantly blocks the sodium current in HEK-293 cells that over express $Na_V1.2$ sodium channels (>80% blockade at 1 μM). Herein, we present our total asymmetric synthesis of hermitamide B, using three major strategies: Keck allylation for chiral center formation, Johnson-Claisen condensation to set the *E*-olefin, and carbodiimide assisted coupling of lyngbic acid with the appropriate amine to give the final product. Yield was 8% over 7 steps with a >95:5 er.

THE IDENTIFICATION OF NOVEL RAT NITRIC OXIDE SYNTHASE 1 FIRST EXONS MAY LEAD TO A BETTER UNDERSTANDING OF DIABETES. Robert L. Murphy, Divya Bansal, Robert Lera, & Terrie K. Rife, Dept. of Biol. James Madison Univ., Harrisonburg VA 22807. The misregulation of nitric oxide synthase I (*NOS1*) has been linked to type-2 diabetes. Due to the difficulty of obtaining human tissue and controlling for environmental influences, many researchers use the rat model to study transcriptional changes in *NOS1* during the pathogenesis of these diseases. However, the rat *NOS1* gene has not been completely characterized, which prevents us from understanding which promoters might be directing disease-related changes in *NOS1* expression. The better-characterized human *NOS1* gene has twelve first exons with associated promoters (1a -1l). The translation start site of the gene is found in the

second exon, which is common to each transcript resulting in the same functional protein. The rat *NOS1* gene has known orthologs to the human first exons 1b, 1c, 1f, and 1g. However, these exons and associated promoters alone do not explain the changes in *NOS1* expression that occur in rats with type-2 diabetes. Thus, rat orthologs to the other human first exons were hypothesized using genomics techniques. Two of the predicted first exons, orthologs to human 1h and 1k, were confirmed to initiate transcription in rats using reverse transcription, PCR, and Southern blotting and were then sequenced. The ortholog to human 1h was found to be expressed in the brain, intestine, kidney, retina and testis, but not in skeletal muscle, and the ortholog to first exon 1k was only expressed in brain. The next step of this research is to study how the transcription of these first exons is altered in diseased rats. This research was funded by a National Biological Honor Society research grant.

RESPONSIVE CHANGES TO NEONATAL GENISTEIN AND ESTRADIOL EXPOSURE IN THE POST-PUBERTAL MOUSE TESTIS AND EPIDIDYMIS: HISTOLOGICAL AND CELLULAR ANALYSIS. Nathan T. Derstine, Ben K. Ruth, & Roman J. Miller, Department of Biology, Eastern Mennonite University, Harrisonburg VA 22802. Effects of neonatal exposure to genistein or estradiol on the post-pubertal testis and epididymis were examined in Swiss mice,injected subcutaneously with control (no-hormone vehicle), estradiol (15µg / injection), or genistein (166µg / injection) every other day from post-natal day (PND) 2 through 14. Mice were necropsied on PND 39; testes and epididymides were prepared for histology. Compared with control, testis weights were reduced in genistein and estradiol groups by 19% and 39% respectively. Seminiferous tubules of genistein and estradiol mice had reduced percentage of tubular wall, total lumen space, and luminal spermatozoa compared to control. In the estradiol group the epididymis organ weightwas reduced by 43% from control and the mean tubular diameter was significantly increased by 7.3%. Spermatozoa counts were reduced by 74% in genistein-treated mice and were not found in the estradiol group. Numerous unusual cells were found in the epididymis tubular lumen of genistein mice. The unusual cells in the lumen are postulated to be immature sperm cells based on the herniated appearance of the seminiferous tubular wall and their close resemblance to primary and secondary spermatocytes. (Research supported in part by Daniel B. Suter Biology Program Endowment, Eastern Mennonite University.)

MURINE FERTILITY FOLLOWING NEONATAL EXPOSURE TO GENISTEIN AND ESTROGEN. Katrina J. Lehman, Jackson T. Maust, Brittany D. Kropf, Kristina R. Landis, and Roman J. Miller, Department of Biology, Eastern Mennonite University, Harrisonburg VA. To determine the effects of selected estrogens on the murine reproductive system, fertility assays were conducted following seven neonatal subcutaneous injections of control vehicle, genistein, or estradiol on postnatal days 2, 4, 6, 8, 10, 12, and 14. Experimental male mice (six control, four genistein, four estradiol) from the treatment groups were individually housed with non-injected control (NIC) female mice as young adults for ten days (postnatal days 58 through 68 ±2 days) to allow mating. Similarly, five control, five genistein, and five estradiol female mice

from the experimentally treated groups were individually housed with NIC male mice for the same time period to allow mating. After the ten days, male mice were removed from the cages. The numbers of mice per litter were recorded on the dates of parturition, about 21-25 days following the mating period. Litter weights and numbers were recorded again on days 15-19 following parturition. None of the injected males in the estradiol group produced any offspring. For the females, statistically significant reductions in fertility were observed in both the genistein-injected females and the estradiol-injected females, although one female mouse from each treatment group produced offspring. The genistein-injected male mice did not appear to have a significant reduction in fertility. Because phytoestrogens such as genistein are found in soy products, continued research is necessary to determine the effects of exposure on fertility and reproductive development. (Research supported in part by Daniel B. Suter Biology Program Endowment, Eastern Mennonite University.)

THE ROLE OF NEURONAL NICOTINIC ACETYLCHOLINE RECEPTORS IN THE ACUTE EFFECTS OF ALCOHOL. Anton J. Dawson, M. Imad Damaj. Department of Pharmacology and Toxicology, Virginia Commonwealth University, Richmond, VA. Tobacco and Alcohol are the two most commonly abused drugs in the world and there is high co-morbidity of addiction to these substances. Substantial evidence suggests that neuronal nicotinic acetylcholine receptors (nAChRs), the receptors to which nicotine binds, may contribute to certain aspects of alcohol dependence. Such evidence has been demonstrated *in vitro* and *in vivo* in animals and in genetic studies in humans. However, the underlying mechanisms of these interactions are still not well understood. Thus we initiated an investigation to uncover the specific nAChR subtypes involved in some of the many underlying mechanisms of ethanol-induced behaviors. We used a variety of approaches to investigate the modulating effects of nicotinic antagonists on such behaviors in C57BL/6J mice. The first approach was to study the acute effects of ethanol-induced loss of righting reflex (LORE), anxiolysis, and hypothermia. The results showed that the drugs Mecamylamine, Dihydro-β-Erythrodine, and Varenicline, in addition to knockout mice lacking the β2 subunit, significantly modulated sensitivity to the ethanol-induced LORE response. Varenicline also reduced ethanol-induced anxiolysis, while increasing sensitivity to the hypothermia response. In conclusion, we have added to data in the field suggesting the involvement of β2-containing nAChRs mediating some of the acute effects of alcohol. Future efforts will continue with additional antagonists and gene knockout mice to understand specific subunits including the largely unexplored α5 and α6 subunits and their relation to ethanol behaviors.

OPIOID AND GP-120 INTERACTIVE NEUROPATHOGENESIS IN HIV-1: ROLE OF CASPASE-3. Kimberly, L. Samano & Kurt F. Hauser. Department of Pharmacology and Toxicology, Virginia Commonwealth University, Richmond VA 23298. gp120, an HIV-1 coat protein, is an established neurotoxin and it is required for viral entry and infection, and its extracellular actions are toxic to microglia, astrocytes, and neurons. It is hypothesized that morphine will exacerbate gliosis and neuronal cell death caused by gp120 *in vivo* and this neuropathogenesis is proposed to occur through

a caspase-3 dependent mechanism. Studies will investigate the effect of morphine and HIV-1 on reactive gliosis of astrocytes and microglia via stereotaxic intrastriatal injection of gp120 into C57BL/6J and caspase 3 knockout (KO) mice. Gliosis will be assessed by co-localization studies performed via fluorescent microscopy probing for glial fibrillary acidic protein (GFAP) and μ opioid receptor as well as Iba_1 with 3-nitrotyrosine (3-NT for nitrosative stress). It is anticipated that μ opioid receptor expressing glia will be more vulnerable to insult versus cells lacking the receptor. Neuronal death will be verified by TUNEL assay to determine if morphine potentiates gp120-induced neuronal apoptosis and to explore if this effect can be alleviated through genetic deletion of caspase-3. Unexpectedly, preliminary results show that morphine administered to caspase-3 KO mice was lethal. This effect was abolished by pretreatment with naltrexone, strongly suggesting this interaction is opioid receptor mediated. Additional studies will be conducted to assess this novel finding and to investigate the mechanism(s) of morphine lethality. Collectively, these studies will add to the understanding of how morphine influences the neuropathogenesis of HIV-1 as well as explore the role of caspase-3 in this interactive comorbidity.

NAVIGATING THE MURKY WATERS OF NICOTINE AND ANXIETY: CONTRIBUTIONS OF THE ß2 SUBUNIT. Shawn M. Anderson & Darlene H. Brunzell. Virginia Commonwealth University School of Medicine, Richmond VA 23298. Nicotine, the primary psychoactive agent in tobacco, exerts its effects by binding to nicotinic acetylcholine receptors (nAChR) in the brain, with ß2 containing nAChRs (ß2*nAChRs) having the highest binding affinity for nicotine. ß2*nAChRs are significantly upregulated in the brains of smokers and rodents following extended exposure to nicotine. Although smokers report that they smoke to relieve anxiety, controlled studies suggest that repeated exposure to nicotine increases anxiety behavior. The purpose of these studies was to assess the role of ß2*nAChRs in anxiety-like behavior. Male ß2*nAChR knockout (?2KO) mice on a C57BL6 background and their wildtype (WT) counterparts were tested in a light-dark box assay after i.p. injection of 0, 0.01, 0.05, 0.1, or 0.5 mg/kg nicotine in 0.9% saline. ANOVA tests revealed a significant interaction of treatment and genotype for behavioral measures of latency and light-chamber exploration. WT animals that received 0.5 mg/kg nicotine showed significant increases in latency to leave dark chamber ($p < 0.05$) and decreases in locomotor activity in the light chamber ($p < 0.05$) compared to controls. These effects were not seen in ?2KO animals. These data suggest that ß2*nAChRs contribute to movement/exploratory behavior in an aversive environment. Consequently, it appears that ß2*nAChRs contribute to the anxiogenic effects of nicotine administration in the light-dark assay and suggest a potential mechanism for elevated anxiety behavior in smokers. This work was supported by a Jeffress Memorial Trust research grant J-951 and a NIDA small grant project award DA005274.

THE FULL AGONIST WIN55, 212-2 EXERTS GROWTH INHIBITORY EFFECTS THROUGH A CANNABINOID RECEPTOR INDEPENDENT MECHANISM. Sean M. Emery, David A. Gewirtz & Aron H. Lichtman, Virginia Commonwealth University, Richmond VA. Cannabinoids are known to inhibit the growth of a variety

of cancer cells in vitro, including those derived from glioma, breast, prostate, and lung tumors, among others. In the present study, we tested whether WIN55, 212-2 (WIN2), a synthetic cannabinoid that acts as a full agonist at both known cannabinoid receptors, CB_1 and CB_2, produces antiproliferative effects in both human (MCF-7 and MDA-MB-231) and murine (4T1) breast tumor cells. WIN2, but not its inactive stereoisomer WIN55, 212-3 (WIN3), elicited inhibitory effects on cancer growth, suggesting that these antiproliferative effects are due to the drug acting at a specific site of action. Interestingly, the highly selective CB_1 and CB_2 antagonists, Rimonabant and SR144528, respectively, did not block the effects of WIN2, either alone or in combination. Instead, each cannabinoid receptor antagonist enhanced the growth-inhibitory actions of WIN2 in each cell line. Both CB_1 and CB_2 are G protein coupled receptors (GPCRs), predominantly activating Gi/Go subtypes. Accordingly, we tested whether pertussis toxin, which inhibits Gi/Go proteins, would prevent WIN2's antiproliferative actions, but the treatment was unable to prevent WIN2's actions in any of the breast cancer cell lines. Taken together, these data suggest that WIN2 inhibits breast cancer cell growth through a non-GPCR mechanism of action, but the stereoselective-dependent nature of this effect suggests specific site(s) of action. Experiments are currently underway to determine the underlying mechanism of action for the antiproliferative effects of WIN2.

MODULATION OF GLIAL FUNCTION BY MORPHINE AND THE HIV-1 PROTEIN GP120. Elizabeth M. Podhaizer & Kurt F. Hauser, Dept. of Pharmacology and Toxicology, Virginia Commonwealth Univ., Richmond VA 23298. Opioid abuse, through injection drug use is tightly linked to HIV-1 infection through both the spread of the disease and by exacerbation of disease progression culminating in HIV-1 encephalitis (HIVE) and neurocognitive impairments. CNS glia are intimately involved in the dual effects of opioids and HIV-1, as glia, unlike neurons, are infected by HIV-1 and additionally release inflammatory and modulatory substances that can activate neighboring glia as well as interfere with neuronal function. Previous work has shown that astrocytes are directly involved in morphine's toxicity. Thus, we hypothesized that opioids acting directly through astrocytes, dysregulate glial function and lead to interactive neurotoxicity in the presence of HIV-1 infection. To address this hypothesis, we examined important convergent signaling events of two $G_{i/o}$ coupled receptors, MOPr and CCR5, the receptors for morphine and the envelope glycoprotein, gp120 respectively. Opioids and HIV-1 proteins elevate intracellular ROS in astrocytes as well as promote increases in intracellular calcium levels independently, which are linked to inflammatory signaling. Morphine, but not gp120 altered p-ERK levels with morphine decreasing p-ERK over a 60 minute period, suggesting that opioids impair the proliferative function of astrocytes. Additionally, agonist selectivity is present between gp120 and the endogenous ligand to CCR5, RANTES which elevated p-ERK over 60 minutes. These results suggest that signaling through opioid and chemokine receptors by morphine and gp120 have similar points of convergence where interactive signaling will be examined.

ARTEMIS ENDONUCLEASE: A CRITICAL REINFORCEMENT TO THE G1 DNA REPAIR ARMAMENT. Susovan Mohapatra[1], L.F. Povirk[1], Imran Khan[2], M.K.Stillion[2] & S.M. Yannone[2], [1]Dept. of Pharmacology and Toxicology, Virginia Commonwealth University, Richmond Va and [2]Dept. of Genome Stability, Lawrence Berkeley Laboratory, Berkeley, Ca. DNA double strand breaks (DSB) are the most significant lesions resulting from radio/chemotherapeutic intervention of cancer. Nonhomologous end-joining (NHEJ) is considered to be a critical DNA repair pathway and mutations in the NHEJ factor Artemis have been implicated in radiosensitive severe combined immunodeficiency (RS-SCID) or Athabascan SCID in humans, as well as increased risk of lymphoma in some settings. Prior *in vitro* studies showed that Artemis has a DNA-PK-dependent endonuclease activity at DNA ends. To assess the possible role of this endonuclease activity in chemo/radioresistance, patient-derived Artemis-deficient CJ179 fibroblasts were stably complemented with lentiviral vectors expressing either wild-type or D165N Artemis, a mutation that eliminates its endonuclease activity. As determined by clonogenic survival assays, expression of wild-type Artemis but not D165N mutant conferred approximately two-fold resistance to ionizing radiation, as well to the radiomimetic agents bleomycin and neocarzinostatin in CJ179 cells. Measurements by γ-H2AX, 53BP1 focus formation and pulse-field gel electrophoresis (PFGE) assays suggested a repair defect (10-20%) in Artemis-deficient and D165N Artemis mutant cell lines, but not in wild-type Artemis-complemented cells, particularly at 6-18 hr post-irradiation. These results, combined with previous *in vitro* studies, suggest that resolution of terminally blocked DNA ends by the endonuclease activity of Artemis may be its biologically relevant function.

GENOMIC ANALYSIS OF THE ETHANOL DEPRIVATION EFFECT. Jonathan A Warner & Michael F. Miles, Department of Pharmacology and Toxicology, Virginia Commonwealth University, Richmond, VA. The potential for relapse into abusive modes of substance use is of paramount concern in recovery from any type of substance addiction. Alcoholism presents significant challenges to the addict due to its strong heritability and the pervasive legal availability of alcohol in most industrialized societies. The ethanol deprivation effect (EDE), also known as the alcohol deprivation effect, models relapse behavior of human alcoholics, and manifests in mice as an increase in ethanol consumption and preference following forced abstinence, which is attenuated by naltrexone and acamprosate. Several transcripts previously identified by microarray analysis of nucleus accumbens as significantly regulated by ethanol deprivation were confirmed with quantitative PCR. These transcripts code for proteins involved in a diverse range of cellular functions, including calcium regulation, mitochondrial localization, RNA interference, and chromatin modification. Because repeated deprivations have been shown to increase the magnitude of the EDE, a long-term “binge” model with repeated deprivations was used to obtain further samples for genomic analysis. After one month of habituation and one month of uninterrupted ethanol access the mice were subjected to eight cycles of six days of ethanol deprivation followed by one day of reinstatement. This model produced a significant and sustained increase in ethanol consumption and preference for ethanol over water following the

first deprivation period, and it should prove useful in further exploration of the molecular mechanisms of both development and maintenance of the EDE, as well as in testing long-term efficacy of therapeutics for alcoholism.

PAIN-RELATED DEPRESSION OF INTRACRANIAL SELF-STIMULATION IN RATS: EFFECTS OF THE DELTA OPIOID AGONIST SNC80 AND THE PSYCHOMOTOR STIMULANT COCAINE. Marisa B. Rosenberg[1], John E. Folk[2], Kenner C. Rice[2] & S. Stevens Negus[1], [1]Deparment of Pharmacology and Toxicology, Virginia Commonwealth University, Richmond VA; [2]Chemical Biology Research Branch, NIDA/NIAAA, Rockville, MD. Pain is associated with a stimulation of some behaviors (e.g. withdrawal responses) and a depression of other behaviors (e.g. feeding, locomotion, responding maintained by many types of positive reinforcement). We have argued that analgesic drug development may benefit from complementary evaluation of drug effects on both pain-stimulated and pain-depressed behaviors. In this study, intraperitoneal injection of dilute lactic acid (1.8% in a volume of 1ml/kg) was used as a noxious stimulus in rats to stimulate a stretching response and to depress intracranial self-stimulation (ICSS) of the median forebrain bundle. The delta opioid agonist SNC80 (1-10 mg/kg, i.p.) dose-dependently blocked both acid-stimulated stretching and acid-induced depression of ICSS without altering control ICSS in the absence of the noxious stimulus. In contrast, cocaine (1-10 mg/kg i.p.) blocked acid-induced depression of ICSS only at doses that also facilitated control ICSS, and cocaine had no effect on acid-stimulated stretching. Flupenthixol (0.01-1 mg/kg, i.p.) blocked acid-stimulated stretching but also decreased control ICSS and only exacerbated acid-induced depression of ICSS. Thus, the antinociceptive effects of SNC80 could be dissociated from the non-selective stimulant effects of cocaine and the non-selective depressant effects of flupenthixol. Supported by NIH grants RO1-DA11460 and R01-NS070715.

ROLE OF INSULIN RESISTENCE IN PCOS AND IMPLICATIONS FOR DEVELOPMENT OF METABOLIC SYNDROME AND HEPATOSTEATOSIS. Aybike Birerdinc[1,2], Nandita Niranjan[1], Noreen Hossain[2,3], Arian Afendi[2,3], Vikas Chandhoke[1,2], Ancha Baranova[1,2] & Zobair Younossi[1,2,3], [1]Molecular and Microbiology Dept. and Center for the Study of Genomics in Liver Diseases, George Mason Univ., Fairfax, VA 22030, [2]Translational Research Institute, Inova Health System, Falls Church, VA 22042 and [3]Center for Liver Diseases, Inova Fairfax Hospital, Falls Church, VA 22042. Polycystic ovarian syndrome is a common disorder observed in women aged mainly in the reproductive age bracket: from 12- 45 years. PCOS is also associated with a number of other pathological features such as obesity, insulin resistance, and disregulation in lipid metabolism. In this study we attempt to determine the genetic commonalities between PCOS and NAFLD as both disorders have the hallmarks of Metabolic Syndrome. For this study 12 patients with diagnosed PCOS and 12 patients with confirmed lack of PCOS were selected. The two cohorts were carefully matched for clinical parameters such as presence of liver disease, BMI and age. These cohorts will be profiled by qPCR arrays to determine gene expression, by ELISA assays

to determine protein abundance, and microRNA panels to determine the involvement of microRNA's.

POSTERS

THE INDUCTION OF HEAT SHOCK PROTEIN 72 AT SPECIFIC HYPOTHERMIC INTERVALS IN AN ISOLATED AND PERFUSED RAT HEART MODEL-IMPLICATIONS FOR CARDIAC TRANSPLANTATION. E. Taylor[1], I. Danelisen[1], V. Sivakumaran[2], J.E. Mahaney[1,2], R.P. Wyeth[1], [1]Via College of Osteopathic Medicine, Virginia Campus, Blacksburg VA 24060 [2]Virginia Polytechnic Institute and State University, Blacksburg VA 24060. Maintaining cardiac viability is a significant determinate in, and limitation to, cardiac transplantation. Current technology restricts the use of donor hearts due to increasing injury suffered to explanted hearts as time to implantation increases. Thus, the time span from procurement to implantation is greatly limited (~4 hours). Heat shock proteins (HSPs) contribute to cellular survival. Understanding HSP's role in cooling and rewarming of explanted hearts may increase the allowable time from explantation to implantation by protecting vital cardiac proteins from denaturation during transplantation. To test this hypothesis, male and females rat hearts were cannulated, stabilized, and perfused for 30 min at target temperatures. Caspase and HSP 72 were quantitated from myocardial homogenates. Myocardial injury, as caspase expression, was greater at the extremes of thermal stress as was myocardial protection, as HSP 72 expression. Furthermore, females expressed significantly more HSP 72 than males at hypothermic conditions. Our preliminary data suggest that HSP 72 is induced by thermal stress and that females are more capable of preventing protein denaturation at hypothermic conditions than are males.

A RETROSPECTIVE STUDY OF THE DISPARITY OF HEALTHCARE IN SOUTHWEST VIRGINIA BASED ON THE PRESENTATION OF ACUTE MYOCARDIAL INFARCTION AND ASSOCIATED MORTALITY IN PATIENTS LESS OR EQUAL TO 50 YEARS OF AGE. E. Taylor[1], J. Powers[1], R.P. Wyeth[1], National Center for the Analysis of Healthcare Data[1], Laboratory for Interdisciplinary Statistical Analysis[2], [1]Via College of Osteopathic Medicine, Virginia Campus, Blacksburg VA 24060, [2]Virginia Polytechnic Institute and State University, Blacksburg VA 24060. Patients 50 years or less of age from southwestern (SW) were compared to patients residing in the remaining health districts of Virginia presenting with initial myocardial infarction (MI) with and without mortality. A total of 2280 patients were identified. The prevalence of initial nonfatal MI was 48.9/100K in SW Virginia and 37.7/100K in the rest of the Commonwealth. The prevalence of fatal MI was 7.0/100K in SW Virginia and 2.6/100K in the rest of the state. Neither prevalence of fatal nor nonfatal initial MI was statistically significant when SW Virginia patients were compared to patients from the remaining Commonwealth. There were however significant differences within mean household income and the population density. The mean income was $55,846 in SW Virginia as compared to $81,235 in the remaining Commonwealth, while the population density was 95 persons/1000 sq mile within SW Virginia and 258 persons/sq mile within the remaining Commonwealth.