Oral Presentation

Abstract Title:	**Precision biochemical profile of Alzheimer's Disease and APOE Genotype**
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Abstract: The cure for Alzheimer's disease (AD) has remained elusive for more than 20 years. The amyloid hypothesis has led researchers to target amyloid in drug discovery to no avail. It is possible that there are underlying metabolic processes that lead to the deposition of amyloid in brain tissue and that these processes differ based on APOE status. Here we measured the metabolites of AD brains and control brains with and without the APOE4 genotype to understand whether differences are implicated in the underlying disease pathology. The global biochemical profiles of post-mortem human brain tissue was determined using mass spectroscopy. 24 subjects from 4 different cohorts were analyzed: APOE3 Control, APOE3 Alzheimer's disease, APOE4 control, and APOE4 Alzheimer's Disease. Metabolites were quantified using global untargeted metabolomics (HD4) and compared between cohorts using Welch's two-sample t-test. Many metabolites were significantly different between the 4 cohorts. Most notably, AD brain tissue regardless of APOE genotype had increases in products related to metabolic syndrome, mitochondrial dysfunction, and kidney dysfunction compared to control brain tissue. APOE4 AD brain tissue had elevated free fatty acids and altered endocannabinoid metabolism compared to APOE3 AD brain tissue. The different biochemical profiles of the different cohorts suggest that metabolic processes underlie disease pathology. It is possible that precision nutrition could be implemented in order to optimize management depending on disease status and genotype. Future studies with larger sample sizes are needed to confirm whether these metabolites are consistently abnormal in AD and APOE4 human brain tissue.

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Oral Presentation

Abstract Title:	**Genome-Wide Association Study of Brain Arteriolosclerosis**
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Abstract: Brain arteriolosclerosis (B-ASC) is a neuropathology characterized by degenerative wall thickening of the arterioles in the brain. Moderate-to-severe B-ASC is associated with worse memory and global cognitive function in people among the “oldest old” (those aged ≥80 years at time of death) after controlling for other neurodegenerative pathologies, and there are independent neuropathological and clinical predictors of arteriolosclerosis in those aged ≥80 years compared to those younger. In this study, we performed a genome-wide association study on B-ASC on subjects with aged ≥80 years at time of death using neuropathological data from the National Alzheimer’s Coordinating Center Neuropathological Dataset (NIA/NIH Grant U01 AG016976) and genetic data from the Alzheimer’s Disease Genetics Consortium (NIA/NIH Grant U01 AG032984). We performed our analysis using an additive model of inheritance and two phenotypic models: a case-control logistic regression model and an ordinal logistic regression model. Two genetic variants, rs6549072 and rs11928305, achieved genome-wide significance ($p < 5e-08$) in the ordinal logistic regression model, with rs6549072 also achieving genome-wide significance in the case-control model. Both variants are found in the gene FAM19A1 on chromosome 3, which codes for a protein in the neurokine family that mediates mobilization of immune cells in the central nervous system. The FAM19A1 protein product is highly expressed in astrocytes, glial cells which form part of the blood-brain barrier and line small blood vessels in the brain. Our study suggests that a neuro-inflammation pathway may be involved in the development B-ASC among those who die over the age of eighty.

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Oral Presentation

Abstract Title:	**Reduced Cerebral Blood Flow in Aging Adults with Down Syndrome: An Arterial Spin Labeling Study**
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Abstract: Introduction: Adults with Down syndrome (DS) develop extensive Alzheimer disease (AD) neuropathology very early in life, but they also exhibit protective cardiovascular traits like the absence of atheroma and hypertension. We used arterial spin labeling (ASL), a quantitative MRI technique that measures cerebral blood flow, to test the hypothesis that the progression of AD in adults with DS would result in compromised global cerebral blood flow (CBF) despite their otherwise healthy cardiovascular profile. Methods: Adults with DS (n=35, aged 26-65yrs) and age-matched control (n=15) were scanned using a pulsed ASL sequence on a Siemens 3T Prisma as part of an ongoing longitudinal study of aging in DS. Quantitative CBF maps were calculated in mL/100g/min and averaged over the entire brain volume. All subjects were also rated as having minimal, moderate, or severe residual arterial signal (RAS). Results: A plot of global CBF versus age reveals a clustering of DS participants over the age of 54 with drastically reduced CBF values. DS participants older than 54 had a 31% reduction in CBF (32.3± 9.6mL/100g/min) versus younger people with DS (46.7± 6.7mL/100g/min, p=0.011). No such pattern is observed in the control group (young= 45.9± 5.8mL/100g/min, old= 40.9± 4.1mL/100g/min). People with DS over the age of 54 also had a significantly higher proportion of severe RAS scores (50%) vs younger people with DS (7%, p=0.005) or non-DS controls (7%, p=0.016), and prevalence of diagnosed dementia (older DS=60%, younger DS=7% p<0.001, Ctl =0% p<0.001). Conclusion: This study has demonstrated that adults with DS exhibit deficits in perfusion that do not occur until the transition to dementia as opposed to other forms of AD where perfusion deficits precede dementia.

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Center for Clinical and Translational Science

Abstracts

Oral Presentation

Abstract Title:	**Apolipoprotein E4 Alters Astrocyte Fatty Acid Metabolism and Lipid Droplet Formation**
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Abstract: Astrocytes are the primary cell population in the brain to oxidize fatty acids (FAs) and the main producers of CNS apolipoprotein E (apoE). While glucose is the preferred energy substrate for cerebral energetic processes, recent studies have shed light on the importance of FAs as an alternative fuel source. Lipid droplets (LDs) serve as energy rich reservoirs and have been associated with the apoE gene (APOE) and neurodegeneration. The E4 allele of APOE (E4) is the strongest genetic risk factor for the development of late onset Alzheimer's disease (AD). Since E4 carriers and individuals with AD exhibit a state of cerebral lipid dyshomeostasis, we hypothesized that APOE may regulate LD metabolism in astrocytes. We found that under basal and lipid-loaded conditions, astrocytes expressing E4 accumulate significantly more and smaller LDs compared to E3 astrocytes. Accordingly, expression of perilipin-2, an essential protein component of LDs, was higher in E4 astrocytes. We then probed FA metabolism by uptake and oxidation assays and found E4 astrocytes to exhibit decreased uptake of palmitate, and decreased oxidation of exogenously supplied oleate and palmitate. We then measured oxygen consumption rate before and after carnitine-palmitoyl transferase 1 (CPT-1) inhibition, and found E4 astrocytes to consume more oxygen for endogenous FA oxidation and generate more LD-derived metabolites, compared to E3 astrocytes. These findings reflect interesting APOE-associated differences in astrocyte LD formation and FA metabolism, and offer the potential for further studies investigating the link between astrocyte lipid storage, utilization, and neurodegenerative disease as a function of APOE genotype.

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Oral Presentation

Abstract Title:	**Common Mechanisms Contribute to Epilepsy and Tauopathy**
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Abstract: Objective: Neurologic disorders are among the most significant health challenges facing society today. Although different neurologic disorders are often thought to be distinct from one another, evidence suggests similar processes may contribute to pathology in different diseases. Previous studies suggest that common disease mechanisms contribute to the development of epilepsy and tauopathy. The purpose of this study is to better characterize this relationship and explore potential therapeutic avenues to slow disease progress. Methods: This study uses the pilocarpine-induced status epilepticus model of temporal lobe epilepsy to explore the effect of severe seizures on tau pathology. Brains were collected from mice at 6 or 24 hours after induced status epilepticus. Homogenates were analyzed via Western blot to look for changes in tau phosphorylation or activity of two major regulators of tau phosphorylation, GSK3β and PP2A. Results: GSK3β activity increased within 6 hours and remained elevated by 24 hours. PP2A activity initially decreased but returned to normal by 24 hours. These data show that changes in tau phosphorylation dynamics occur at a much earlier time point after status epilepticus than has previously been described. Conclusions: The current project supports previous observations that seizures promote tau phosphorylation in vivo, but suggests that changes begin much earlier than previously thought. Further work is needed to understand how post-seizure changes in tau phosphorylation develop over longer periods of time. Additionally, future work will characterize the effect of tauopathy on electrical activity in vivo and in vivo.

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Poster Presentation 95

Abstract Title:	**Altered Parvalbumin Cell Populations in Dorsolateral Prefrontal Cortex after Neonatal Hippocampal Damage in Macaques.**
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Abstract: The parvalbumin (PV) class of GABA interneurons is involved in sustaining the normal neuron signaling that supports proper functionality of the Dorsolateral Prefrontal Cortex (dlPFC) executive system. Because the dlPFC undergoes a protracted development, any disruption may play a role in working memory impairment and other cognitive deficits associated with neurodevelopmental disorders including schizophrenia (SCZ). Thus, we predicted that a neonatal hippocampal lesion would disrupt the normal PV-positive cell maturation within the dlPFC, consistent with an early limbic-prefrontal disconnection model of SCZ. We used tissue from four control and four experimental adult rhesus monkeys that sustained bilateral neonatal hippocampal lesions of varying degree to better understand the role of the hippocampus in dlPFC development. Specifically, we analyzed the relative density of PV-positive GABAergic interneurons in cortical layers IIIA and IIIB of Brodmann Area 46d of lesioned (Neo-H) and non-lesioned monkeys (Neo-C). We found a significantly higher density of PV-positive cells, specifically within layer IIIA of the dlPFC of Neo-H monkeys relative to the Neo-C monkeys, both between the left hemispheres of the two groups and across the groups when both hemispheres were considered together. These results were confirmed by estimations of total PV-positive cell populations in layer IIIA of area 46d. Moreover, layer IIIA PV densities of the Neo-H group were significantly positively correlated with the extent of hippocampal damage. Ultimately, these findings have implications for how neonatal lesions of the hippocampus may affect vulnerable structures and disrupt cognitive processing, eventually leading to deficits characteristic of neurodevelopmental disorders including schizophrenia.

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Poster Presentation 104

Abstract Title:	**The Bayesian Method for Confounding Adjustment as Applied to Personality and Substance Use Data to Estimate Average Causal Effect**
Author(s):	L. Su, MD/PhD program, University of Kentucky C. Wang, Department of Biostatistics, University of Kentucky C. Lee, Department of Psychology, University of Kentucky R. Milich, Department of Psychology, University of Kentucky D. Lynam, Department of Psychology, Purdue University

Abstract: Purpose: To investigate possible correlations between substance use and personality trait measurements in students attending the University of Kentucky using the Bayesian Adjustment for Confounding. Methods: The analysis was done in the statistical analysis software R using the Bayesian Adjustment for Confounding as developed by Dr. Chi Wang et al. The resulting model related the personality trait measures with substance use while accounting for a multitude of confounders. Data/Results: There were 449 individuals in the data. The dataset contained 10 different personality measurements from two different models. These variables were the exposure variables. The four outcome variables used were frequency of alcohol use, frequency of marijuana use, frequency of tobacco use, and audit total score, a measure of how harmful the subject's alcohol use is. 37 confounders were also included in the model, including sex, race, age, and quite a few variables involving the subject's friends' usage and opinions of alcohol, marijuana, and stimulants. This resulted in evaluating 40 associations/relationships, each relating one exposure variable to one outcome variable. The results showed which confounders were selected often in each model. The average causal effect (ACE) was also calculated from the models, providing a measurement of the actual level of causation between the two variables. Conclusions: Overall, the Bayesian Adjustment for Confounding is a method useful for eliminating confounders in observational studies and establishing causation with more certainty. The relationship that showed the highest positive effect was between positive urgency and audit total score. The relationship showing the most negative effect was between conscientiousness and audit total score. An example of a relationship with no effect was between marijuana use frequency and extraversion. Through the BAC method, the direct effects of personality traits on substance use can be accurately estimated.

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Poster Presentation 108

Abstract Title:	**Building on Appalachian Cultural Traditions to Support Rural Grandparent Caregivers**
Author(s):	M. N. Dunfee, Department of Behavioral Science, U of Kentucky N. E. Schoenberg, Department of Behavioral Science, U of Kentucky R. L. Brown, Department of Sociology, U of Kentucky

Abstract: Purpose: Poverty and poor health disproportionately affect older adults serving as primary caregivers to their grandchildren. Grandparent caregivers living in rural and underserved regions, including Appalachia, are especially vulnerable. However, Appalachian cultural traditions, including religious practices and spirituality, offer grandparents support when facing these challenges. Methods: To improve understanding of the role religion and spirituality play in coping, twenty-six grandparent caregivers, recruited through community organizations and snowball sampling, engaged in a series of four interviews. A coding team applied conventional content analysis to the transcripts, employing multiple approaches to ensure rigor and transferability. Results: Findings suggest that religion and spirituality help grandparent caregivers cope by (1) providing a sense of purpose and perspective; (2) fostering peace and perseverance; (3) encouraging forgiveness; and (4) strengthening social cohesion. Discussion/Conclusion: An improved understanding of the coping strategies employed by grandparent caregivers combined with a greater awareness of existing community assets can inform effective interventions for grandfamilies. Our findings suggest numerous opportunities for practitioners, policymakers, faith leaders, and social service administrators to leverage cultural traditions in Appalachian communities in order to support grandfamilies. For example, acknowledging the great solace grandparents find in attending church, religious and spiritual organizations may consider expanding programming for grandparents through developing programs that facilitate grandparents' development of a sense of purpose through good works, or programs that honor grandparents such as intergenerational scripture studies. Community leaders, local service providers and spiritual leaders may also consider seeking opportunities to locate programming for grandparents in religious and spiritual locations.

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Poster Presentation 109

Abstract Title:	**Risk Factors for Severe Maternal Morbidity in Kentucky Women**
Author(s):	Anna Hansen, Department of Sociology, University of Kentucky Dr. Svetla Slavova, Department of Biostatistics and Kentucky Injury Prevention Center, University of Kentucky

Abstract: Background. Women in the United States have face a steadily increasing risk of experiencing severe maternal morbidity (SMM) in pregnancy. The purpose of this study is to elucidate what factors increase Kentucky women's risk of experiencing SMM. Methods. We identified obstetric patients in state-wide inpatient hospital data in 2017. We used a logistic regression model to evaluate the association between experiencing SMM, women's demographic information, and common chronic disease states. Results. 2.12% of cases included within the study exhibited SMM. Risk factors significantly associated with SMM were race, state region, rurality and common disease states, namely hypertension, diabetes, and opioid use. When controlling for all other independent variables, the odds of black women experiencing SMM was nearly twice that of non-black women. The odds of Appalachian women experiencing SMM was 1.3 times the odds of non-Appalachian women, and the odds for women living in rural counties not adjacent to metropolitan counties was 1.5 the odds of women living in more populated areas. Common pre-pregnancy disease states associated with poor maternal outcomes in other populations were also determined to increase risk for SMM amongst Kentucky women. The odds of women with hypertension experiencing SMM was 20 times the odds of non-hypertensive women, the odds of a woman with type-II diabetes mellitus was 1.7 that of non-diabetic women, and the odds of women with a history of opioid use was twice that of women with no history of opioid use.

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Poster Presentation 116

Abstract Title:	**A Protein Tyrosine Phosphatase 4A3 (PRL-3)/Wnt Signaling Axis as a Novel Therapeutic Target in Acute Lymphoblastic Leukemia (ALL) Relapse**
Author(s):	M. G. Haney, Department of Molecular and Cellular Biochemistry, University of Kentucky A. K. O'Leary, Department of Molecular and Cellular Biochemistry, University of Kentucky J. S. Blackburn, Department of Molecular and Cellular Biochemistry, University of Kentucky

Abstract: Acute Lymphoblastic Leukemia (ALL) is the most common pediatric malignancy and 15-20% of patients experience relapse, which is frequently more aggressive and treatment resistant than primary disease with unfavorable outcomes. Relapse occurs because conventional chemotherapies are unable to reliably and completely eliminate leukemia stem cells (LSCs), which have the ability to self-renew and form a leukemia from a single cell. The Wnt signaling pathway has emerged as having an important role in LSC self-renewal in T-ALL, but current Wnt inhibitors have unacceptable toxicity in the clinic. I have found the Protein Tyrosine Phosphatase 4A3 (PTP4A3 or PRL3) is highly expressed by ALL cells that also express Wnt pathways genes, and is not expressed by normal cells. In a zebrafish Myc-induced ALL model, PRL3 expression significantly enhanced LSC frequency, while inhibition of PRL3 reduced LSC numbers in vivo. In human cells, I found that PRL3 activates the expression of Wnt pathway genes. I have created transgenic zebrafish models of ALL that over-express both wild-type and mutant forms of PRL3 and constitutively active beta-catenin to define the role of PRL3 in Wnt signaling by assessing the effects of PRL3 and PRL3 mutants on LSC self-renewal and the phosphorylation status of Wnt pathway components in zebrafish models and human ALL cells. My research defines a novel role for the phosphatase PRL3 in self-renewal of cancer stem cells via activation of Wnt signaling, and targeting PRL3, which is expressed specifically by leukemia cells, represents a novel therapeutic strategy to inhibit WNT signaling in ALL.

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Poster Presentation 124

Abstract Title:	**Photothrombotic microinfarct technique for chronic, in vivo imaging of mouse vasculature and astrocyte networks using multiphoton microscopy**
Author(s):	N. Farr, Sanders-Brown Center on Aging, U of Kentucky P. Sompol, Sanders-Brown Center on Aging, U of Kentucky J. Gollihue, Sanders-Brown Center on Aging, U of Kentucky I. Artiushin, Sanders-Brown Center on Aging, U of Kentucky C. M. Norris, Sanders-Brown Center on Aging, U of Kentucky

Abstract: Vascular pathology contributes significantly to cognitive aging. Given the high incidence of cardiovascular disease in Kentucky and nationwide, vascular contributions to cognitive impairment and dementia (VCID) are a leading cause of dementia. The mechanisms by which vascular disease changes the brain are still largely unknown. Multiphoton microscopy helps by showing both the structure and the physiology of vessel-astrocyte interactions. Vascular pathology is modeled in our lab by delivering a precise infarct using LASER-activated thrombosis. Stereotaxic coordinates allow for precise placement of thrombosis, such as in the hippocampus or barrel cortex. Blood flow dynamics can then be measured at various time intervals, such as 30 minutes, one hour, six hours, and 48 hours after the insult. Ablation is directed and timed to minimize collateral damage to the surrounding tissue as well as potential vasogenic and cytotoxic edema formation. Adeno-associated virus injection of GCaMP6 allows for visualization of calcium signaling as astrocytes respond to infarction. Application of this technique in amyloidogenic APP/PS1 mice promises to illuminate the convergence of Alzheimer's disease (AD) and VCID related pathologies. Capillary flow stalls have also been observed with this technique in wild-type mice in the absence of infarction, sparking the question of the frequency and duration of stalls, as well as how that may be different in models of AD.

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Poster Presentation 125

Abstract Title:	**AT1a Receptor Deficiency Attenuates Thoracic Aortic Aneurysm Progression in FBN1 C1041G/+ Mice**
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Abstract: Angiotensin receptor type 1 (AT1 receptor) activation has been implicated in thoracic aortic aneurysms (TAAs). Losartan, an AT1 receptor antagonist, attenuates TAAs in multiple animals models. Recent studies concluded that losartan's attenuation of Marfan syndrome associated TAAs is unrelated to AT1 receptor antagonism. We determined the effects of AT1a receptor deletion on TAAs in the fibrillin-1 haploinsufficient (FBN1 C1041G/+) Marfan syndrome mouse model. Aortas from wild type and FBN1 C1041G/+ littermates, that were AT1a receptor +/+ or -/-, were imaged from 1 to 12 months of age using a rigorously standardized ultrasound protocol and verified by direct visualization at termination. Male FBN1 C1041G/+ mice had increased aortic diameters at 1 month compared to wild type littermates (Ascending: 1.39±0.06mm vs 1.16±0.07mm; p=0.04. Root: 1.63±0.05mm vs 1.35±0.06mm; p<0.001). Dilation at 1 month was not attenuated by AT1a receptor deletion. Subsequent expansion of both the ascending aorta and the aortic root in male FBN1 C1041G/+ mice was attenuated by AT1a receptor deletion. This difference in FBN1 C1041G/+ mice with AT1a receptor +/+ vs -/- could be detected at 3 months (Ascending: 1.51±0.04mm vs 1.28±0.06mm; p=0.002. Root: 2.05±0.06mm vs 1.79±0.08mm; p=0.03) and persisted to termination. Conversely, aortic diameters in 12 month old female FBN1 C1041G/+ mice compared to their wild type littermates were minimal (Ascending: 1.50±0.06mm vs 1.36±0.06mm. Root: 2.06±0.13mm vs 1.77±0.13mm). Deletion of AT1a receptors attenuates TAA progression but not initial development in male mice. Minimal aortic expansion in female FBN1 C1041G/+ mice highlights the need to perform sex-specific analyses of TAAs.

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Poster Presentation 131

Abstract Title:	**Anti-apolipoprotein A-I Antibody Profiles Correlate with Cardiovascular Disease Outcomes**
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Abstract: Apolipoprotein A-I (ApoA-I) is a target of IgG autoantibody induction in patients, but the role of these antibodies has not been fully elucidated. Anti-ApoA-I IgG antibodies targeting delipidated ApoA-I have been characterized as a biomarker of cardiovascular disease progression, but only moderate associations have been reported. We hypothesize that antibodies bound to ApoA-I as an immune complex are a critical and unexplored component of the antibody response to ApoA-I. An ELISA assay was used to screen plasma from 359 patients with coronary artery disease (CAD). Analysis of outcomes shows that patients in the lowest tertile for ApoA-I/IgG IC values have an increased risk for death and non-fatal myocardial infarction as compared to patients in the highest tertile with an hazard ratio of 1.89 (95% CI: 1.02-3.52; p = 0.04) after adjustment for 6 common cardiovascular risk factors. Pearson correlation analysis between ApoA-I/IgG ICs in the found no relationship between ApoA-I/IgG ICs and 26 common clinical measures. The antibody subclass composition of ApoA-I/IgG IC were then characterized in a second cohort of healthy blood donors and found to be enriched in IgG4. The ratio of pro-inflammatory IgG1 and anti-inflammatory IgG4 were compared between total plasma (9.9 IgG1/IgG4) and within the immune complex (0.30 IgG1/IgG4, p = 0.0003). The enrichment in the anti-inflammatory IgG4 provides a potential mechanisms of the protective effect of ApoA-I/IgG ICs in patients. The identification and further characterization of ApoA-I/IgG ICs has the potential to guide clinical diagnosis and intervention strategies in patients with atherosclerotic cardiovascular disease.

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Poster Presentation 139

Abstract Title:	**FND 4b Decreases Proliferation and Increases Apoptosis of Triple Negative Breast Cancer through AMPK Activation**
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Abstract: Purpose: Triple negative breast cancer (TNBC) is the most lethal and aggressive subtype of breast cancer. AMP-activated protein kinase (AMPK) is a major energy regulator that suppresses tumor growth, and 1-(3-chloro-4-((trifluoromethyl)thio)phenyl)-3-(4-(trifluoromethoxy)phenyl)urea (FND-4b) is a novel AMPK activator that inhibits growth and induces apoptosis in colon cancer. The hypothesis was that FND-4b would reduce growth and induce apoptosis of TNBC through AMPK activation. Methods: (i) Estrogen-receptor positive breast cancer (ER+BC; MCF-7 and T-47D), TNBC (MDA-MB-231 and HCC-1806), and breast cancer stem cells were treated with FND-4b for 24h. Immunoblot analysis assessed AMPK, acetyl-CoA carboxylase (ACC), ribosomal protein S6, cyclin D1, and cleaved PARP. (ii) Proliferation was assessed by performing sulforhodamine B growth assays and cell counting assays after 72h of FND-4b treatment. (iii) Cell death ELISA assays were performed after treating ER+BC and TNBC cells with FND-4b for 72h. Results: FND-4b increased AMPK activation with concomitant decreases in ACC activity, phosphorylated S6, and cyclin D1 in all subtypes. FND-4b decreased proliferation in all cells, while dose-dependent growth decreases were found in ER+BC and TNBC. Increases in apoptosis were observed in ER+BC and the MDA-MB-231 cell line with FND-4b treatment. Conclusions: Our findings indicate that FND-4b decreases proliferation for a variety of breast cancers by activating AMPK and has notable effects on TNBC. The growth reductions were mediated through decreases in fatty acid synthesis, mTOR signaling, and cell cycle flux. ER+BC cells were more susceptible to FND-4b-induced apoptosis, but MDA-MB-231 cells also underwent apoptosis with higher dose treatment.

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Poster Presentation 142

Abstract Title:	**Characterizing unique regulatory sequences in sterol biosynthetic enzymes for the control of fungal pathogens**
Author(s):	K. B. Linscott, College of Medicine, U of Kentucky J. Chappell, Department of Pharmaceutical Sciences, U of Kentucky

Abstract: Invasive fungal infections are a significant cause of patient morbidity and mortality, indicating a need for the identification of new therapeutic targets. Squalene synthase is the first committed step in sterol biosynthesis, and while this enzyme plays a critical role in cell growth, the protein architecture is shared among eukaryotes and so is resistant to the design of fungal-specific growth inhibitors. It has been shown that there is a unique component of the fungal carboxy-terminal domain which allows the fungal squalene synthase, not the enzyme from plants or animals, to complement a knockout mutation in yeast. We hypothesize that there is a fungal-specific motif within this domain involved in regulation of the sterol pathway that can be mimicked for the development of an antifungal therapeutic. To identify this motif, we used the yeast Saccharomyces cerevisiae with a squalene synthase knockout mutation and expressed chimeric squalene synthases originating from multiple kingdoms of life. In contrast to previous observations, all enzymes tested were able to partially complement the knockout mutation when the genes were weakly expressed. Induction of non-fungal squalene synthases could not complement the yeast mutation and led to the accumulation of carboxy-sterol intermediates. These results suggest that the motif is involved in mediating an interaction between squalene synthase and the downstream C4-decarboxylase. Restoration of the complete complementation phenotype was mapped to a kingdom-specific 26-amino acid hinge motif, and over-expression of the C-terminal domain containing this hinge motif from a fungal squalene synthase led to growth inhibition of wild-type yeast.

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Poster Presentation 156

Abstract Title:	**Epithelial-Specific P85α KO Enhances Crypt Resilience to Radiation Injury**
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Abstract: High-dose radiation targets highly proliferative compartments, making radiation an attractive option for aggressive cancers. However, radiation exerts stress on physiologically high cycling cells, including intestinal epithelial cells (IEC), where it causes significant toxicity (diarrhea, bleeding, etc). Here we examine the role of PI3-Kinase (PI3K) signaling in promoting epithelial repair after radiation injury. Previously, we found that reductions in class IA PI3K (pik3r1) (regulatory subunit p85α) induces the anti-apoptotic protein survivin and promotes IEC expansion in an ileocecal resection repair model. Preliminary data obtained in histopathologic sections from radiation proctitis patients reveal a 29.3% enhancement of survivin+ nuclei compared to normal colonic biopsies. To interrogate the role of IEC PI3K in radiation injury, we utilized VillinCre-p85fl//fl (p85KO) and VillinCre-p85+/+ subjected to high dose (12Gy) radiation. IEC Western blot (WB) data of unperturbed p85KO mice revealed a complete ablation of p85α, with subsequent increases in p-AktSer473 along with p-PTEN, p-GSK3βSer9, as well as p-p70S6K and survivin compared to WT controls, suggesting a deregulation of PI3K machinery. RT-PCR studies performed at baseline revealed increases in TA-enriched Wnt target genes, Axin2 (56%) and c-myc (39%) and reserve intestinal stem cell (ISC) markers HopX (33%), and Bmi1 (20%), at the expense of the active cycling Lgr5+ stem cells (-25%). Histopathologic sections highlight a distinct shift in the zone of proliferation with more than a 2-fold increase in BrdU+ cells at the reserve stem cell position 4 compared to controls (Fig 1). Following lethal radiation dosage, p85KO mice exhibited a 20% increase in survival as compared to wildtype (WT) littermates along with increased crypt survival (proportion of crypts with >5 BrdU+ cells/crypt, WT vs p85KO: 72% +/- 3 Vs 84% +/- 1, $p<0.004$). IEC markers of Akt activation (pGSK3β) increase from 24 to 84hr post radiation injury along with markers of activated stem cells (p-β-catSer552, p-PTEN). In p85KO mice, radiation induced lower levels of WB PUMA and cleaved caspase 3 compared to WT controls. Concomitantly, crypt lengths increased in p85KO (+9%) compared to WT (-20%). Taken together, our data suggest PI3K signaling enhances recovery from radiation injury through expansion of reserve ISC populations capable of creating proliferative Lgr5+ ISC and accelerating crypt IEC recovery from radiation-induced cell death. We posit this pathway limits apoptosis and enhances survival of proliferating progenitor populations which increases overall crypt survival. Given results suggesting p85α KO IEC increase PI3K signaling, we propose p85α as a potential drug-able target capable of enhancing recovery from radiation therapy.

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Center for Clinical and Translational Science

Abstracts

Poster Presentation 163

Abstract Title:	**The altered neonatal CD8 T cell immunodominance hierarchy to influenza virus antigens impacts peptide vaccination.**
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Abstract: Neonates are more susceptible to influenza virus infection than adults, resulting in increased morbidity and mortality as well as delayed clearance of the virus. Vaccination continues to be the most important intervention for preventing Influenza disease, but current vaccines fall short of full protection and must be readministered every year. Work is underway to not only stimulate antibody responses to the virus but also promote CD8 T cell responses. CD8 T cells can provide heterosubtypic protection year to year as well as reducing morbidity for infections that do occur. This may be important for improving outcomes in vulnerable populations such as neonates, but neonatal T cells frequently respond differently than adult cells. We sought to understand CD8 T cell specificity and immunodominance during neonatal influenza infection and peptide vaccination as well as how any differences from the adult hierarchy might impact peptide vaccine effectiveness. We found that neonatal C57BL/6 mice display an altered CD8 T cell immunodominance hierarchy during influenza infection, preferentially responding to an epitope in the influenza protein PA rather than the co-dominant adult response to NP and PA. Similarly, pups respond to PA but not NP during peptide vaccination. These data suggest that vaccines targeting T cells should consider epitope usage if the intended patient population includes infants as well as adults.

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Poster Presentation 168

Abstract Title:	**Untargeted Lipidomics of NSCLC Shows Differentially Abundant Lipid Classes in Cancer vs Non-Cancer Tissue**
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Abstract: Lung cancer is the leading cause of cancer death worldwide and non-small cell lung cancer (NSCLC) represents 85% of newly diagnosed lung cancers. The high mortality rate of lung cancer is due in part to the lack of effective treatment options for advanced disease. A major limitation in the development of effective treatment options is our incomplete understanding of NSCLC metabolism at a molecular level. Improvements in mass spectrometry combined with our untargeted assignment tool SMIRFE enable the systematic and less biased examination of NSCLC metabolism. From 86 patients with suspected resectable stage I or IIa primary NSCLC, lipid extracts were prepared from paired disease and non-disease tissue samples and analyzed using ultra-high resolution Fourier transform mass spectrometry. Machine learning was employed to classify SMIRFE formula assignments into lipid categories with which differential abundance analysis was performed. Sterols and glycerolipids were consistently and significantly upchanged in disease versus control. This molecular phenotype suggests a possible therapeutic role for statins in the treatment of NSCLC. Additionally, several sterols belonging to the sterol ester subcategory are consistently and significantly upchanged, suggesting increased SCD1 activity. Although statin use and SCD1 expression have known effects on NSCLC prognosis, their metabolic effects are less understood. In our study, a large fraction of the NSCLC samples displayed this phenotype, suggesting that this metabolic phenotype may be shared across multiple genetic subtypes of NSCLC. Thus, pharmaceutical targeting of this metabolic subtype could have utility in the treatment of many genetic subtypes of NSCLC.

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Poster Presentation 172

Abstract Title:	**Implementing Agglomerative Hierarchical Cluster Analysis for Fluid Biomarkers in Dementia Research**
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Abstract: Agglomerative hierarchical cluster analysis (HCA) is a commonly used unsupervised machine learning approach to find natural clusters of patients within a designated cohort. HCA is achieved by calculating a dissimilarity matrix between all points followed by clustering similar points together until eventually all points are grouped within the same cluster. Unfortunately, there is not a one-size-fits-all approach for this technique and key decisions must be made in order to best analyze the dataset at hand. Our goal in this study was to apply HCA methods to biofluid biomarker datasets to determine the optimal analytic procedure and to identify lead biomarker candidates for further clinical application. In this project we analyzed how different distance metrics, linkage types, and clustering techniques affect the produced HCA outputs. We focus on use of the Minkowski distance metric versus the traditional Euclidean distance metric using varied p parameters in calculating the dissimilarity matrix between points and their effect on the adjusted rand index in different distributions of simulated data. Our data suggest that on biofluid biomarker datasets both types of HCA are comparable and produce distinct profiles of disease within a population of patients with mild cognitive impairment due to cerebrovascular disease.

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Center for Clinical and Translational Science

Abstracts

Poster Presentation 174

Abstract Title:	**Automated High-Content Analysis of Skeletal Muscle Immunohistology**
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Abstract: High volume analysis of skeletal muscle histological cross sections is often necessary for studying muscles physiology. As automation improves for immunohistochemistry and fluorescence microscopy, preparation and imaging of muscle sections is performed with ever increasing speed and efficiency. As such, high content image data analysis represents the most significant bottleneck in the workflow, especially for large-scale studies. To date, no fully automated, accurate, and reliable software is yet available to muscle researchers. Therefore, we introduce FiberVision, a software that 1) improves upon previously reported algorithms, 2) achieves >94% accuracy for myofiber detection, size measurement, type classification, and myonuclear counting without human input, and 3) is available with a readily usable interface. FiberVision is the most robust, intuitive and free software available for muscle histological analysis, and will greatly improve analysis efficiency for the spectrum of muscle researchers.

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Poster Presentation 177

Abstract Title:	**Developing Zebrafish Models to Study the Link Between SoxC Transcription Factors and CHARGE Syndrome**
Author(s):	L.A. Krueger, Department of Biology, U of Kentucky A.C. Morris, Department of Biology, U of Kentucky

Abstract: The molecular mechanisms underlying the ocular birth defects observed in CHARGE patients are poorly understood. Our laboratory studies the development of the vertebrate visual system using zebrafish. Previous work from our lab has shown that knockdown of Sox11, a member of the SoxC family of transcription factors, in zebrafish results in microphthalmia, coloboma, brain, trunk, and heart defects, all phenotypes observed in CHARGE syndrome. Furthermore, a duplication of Sox11 has been identified in a patient clinically diagnosed with CHARGE syndrome, and CHD7 has been shown to directly interact with Sox11 and Sox4 in neural stem cells. Taken together, these data strongly suggest that loss of SoxC expression contributes to the ocular and other phenotypes observed in Chd7-associated CHARGE syndrome. In this study, we begin to further investigate the role that Sox11 plays in the phenotypes seen in CHARGE syndrome by generating Sox11-mutant zebrafish using the CRISPR-Cas system. The resulting Sox11 mutant lines will be characterized for phenotypes related to CHARGE and will be compared to an established CHD7 mutant line. These experiments will provide a better understanding of the potential role of Sox11 in the pathogenesis of CHARGE.

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Poster Presentation 178

Abstract Title:	**Neuro-Avatar: A Reverse Translational Model of an Ongoing Cell Therapy Clinical Trial for Parkinson's Disease**
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Abstract: Currently two clinical trials (NCT01833364 and NCT02369003) are underway which feature the implantation of autologous peripheral nerve grafts to the brain (targeted to the Substantia Nigra, Nucleus Basalis of Meynert, or Putamen) in combination with Deep Brain Stimulation (DBS) for the treatment of patients with Parkinson's disease. This nerve tissue is harvested from the sural nerve, a cutaneous sensory nerve located in the lateral ankle, of patients undergoing DBS surgery. The nerve receives a conditioning injury 14 days before grafting, and samples are collected from the pre-conditioned and post-conditioned nerve. RNA sequencing of these nerve samples shows transcriptome changes consistent with the expected pro-regenerative changes of transdifferentiated repair phenotype Schwann cells. However, the neurobiology of the graft within the brain, the regenerative activity of the pre vs post-lesioned nerve, and the survival of grafted tissue have not been examined. In order to address these questions, this study aimed to develop an animal model of the grafting procedure using the same human tissue grafted into patients with Parkinson's disease. Athymic nude (Hsd:RH-Foxn1rnu) rats were stereotaxically implanted with segments of human peripheral nerve (pre-conditioned or post-conditioned) into the dorsal striatum. Each animal received a unilateral graft with a contralateral sham insertion. Two weeks or six months post-implant the brains of these animals were processed for histopathological analyses. Assessment of graft cell survival, graft morphology, and host tissue response will be reported. In summary, this study uses clinical trial samples to answer basic science questions that will guide future clinical trial design.

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Poster Presentation 179

Abstract Title:	**"See Blue. See Through." CLARITY for 3-D In Vivo Imaging of the Neurovascular Unit**
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Abstract: CLARITY is a newly developed tissue clearing method used for the transformation of biological tissue into a tissue-hydrogel hybrid, enabling highly detailed images of the brain's cellular structure. Historically, imaging studies have been limited to small regions of the brain or do not allow for staining of relevant proteins or genes. CLARITY uses an acrylamide hydrogel to maintain the structural organization of proteins and nucleic acids and surfactant-assisted delipidation to render the tissue permeable to immunostaining and suitable for detailed microscopic analysis. For our studies, we used the X-CLARITY™ System from Logos Biosystems. Male CD-1 mice were anesthetized; the thorax was opened; and an infusion needle was placed into the left cardiac ventricle to perfuse the brain with PBS and paraformaldehyde. Whole brain was collected and fixed in paraformaldehyde. After washing with PBS, brains were either processed as a whole or sliced into sections. Brain tissue was placed in hydrogel solution and hybridized utilizing the X-CLARITY™ Polymerization System. Once hybridized, lipids from the tissue were removed through electrophoresis with ionic detergents using the X-CLARITY™ Tissue Clearing System. After clearing, the neurovasculature was stained with collagen IV primary antibody followed by incubation with Cy3-conjugated secondary antibody. In addition, we cleared the brains of mice with YFP-labeled neurons. Cleared brain tissue was imaged using a Nikon A1R inverted confocal microscope. We are currently using CLARITY with single- and two-photon microscopy imaging to examine the spatial relationship between cells of the neurovascular unit in animal models of neurodegenerative and neurological disorders.

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Poster Presentation 181

Abstract Title:	**Experimental Internal Carotid Artery Stenosis Models Pathogenic Features of Moyamoya Syndrome**
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Abstract: Moyamoya is an arteriopathy defined by the progressive stenosis of the intracranial internal carotid arteries accompanied by the formation of abnormal vascular networks. To address the paucity of research on the adult-onset Moyamoya Syndrome, which completely lacks any animal model for evaluation, our lab created a novel surgical technique, termed internal carotid artery stenosis (ICAS), that attempts to model the cerebrovascular changes seen in the human Moyamoya Syndrome. We hypothesized that the ICAS model would mimic the key proposed pathogenic features of Moyamoya Syndrome; namely, intimal hyperplasia of the major vessels of the cerebrovasculature leading to vessel stenosis and the formation of compensatory collateral arteries. ICAS (n=9) and sham (n=6) surgeries were performed in a randomized fashion on male C57Bl/6 mice (age = 16 weeks). RNA was collected 28-days post-surgery. Expression of previously identified genes associated with human Moyamoya Syndrome (VEGF, SDF-1a, MMP-9, bFGF, VCAM-1, and MCP-1) was quantified by qPCR. PRISM software was used for data analysis. ICAS increases genetic expression of VEGF in the whole brain (fold change=0.60, p=0.050) and SDF-1a in the striatum (fold change=0.59, p=0.016), and decreases genetic expression of MMP-9 in the ipsilateral cortex (fold change=0.39, p=0.044). The upregulation of VEGF and SDF-1avis indicative of hypoxia-induced angiogenesis and ischemia-induced inflammation, and the downregulation of MMP-9 may indicate decreased extracellular remodeling in the cortex. Taken together with the previous discovery that ICAS induces stenosis of the major vessels of the cerebrovasculature, these findings support ICAS as a relevant model of the proposed pathology of Moyamoya Syndrome.

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Poster Presentation 182

Abstract Title:	**Network-Dependent Effects of Alzheimer's and Cerebrovascular Pathology on White Matter Decline**
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Abstract: White matter (WM) is affected by both Alzheimer's disease (AD) and cerebrovascular disease (CVD) pathology. Recent work has suggested that CVD and AD pathology independently contribute to white matter in spatially distinct brain regions cross-sectionally. However, it is unknown how AD and CVD pathology differentially impact longitudinal change in WM connections within large-scale brain networks underlying human cognition. Eighty-three non-demented individuals were recruited to investigate how AD and CVD pathology predicted change in baseline and 1-year follow-up diffusion tensor imaging (DTI) data within major brain networks: the default mode network (DMN), executive control network (ECN), dorsal attention network (DAN), and the hippocampal network (fornix). Cerebrospinal fluid (CSF) β-amyloid (Aβ) concentration was used to measure AD pathology and hypertension (HTN) diagnosis was used as a marker of CVD risk. Multiple linear regression analyses that included Aβ and HTN as simultaneous predictors of WM decline in each network indicated that Aβ alone predicted decline in fornix WM, HTN alone predicted decline in ECN WM, both Aβ and HTN predicted decline in DMN WM, and neither Aβ nor HTN predicted decline in DAN WM. These results indicate that AD and CVD pathology differentially affect WM connections in a way that mirrors their predominant cognitive changes. AD pathology preferentially affects fornix and DMN WM, which are both involved in memory. In contrast CVD pathology affects ECN and DMN WM, which are both involved in executive function. Therefore, breakdown in WM connections may be an important mechanism by which these pathologies influences cognition.

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Poster Presentation 238

Abstract Title:	**Comparing effects of CDK inhibition and E2F1/2 ablation on neuronal cell death pathways in vitro and after traumatic brain injury**
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Abstract: Traumatic brain injury (TBI) activates multiple neuronal cell death mechanisms, leading to post-traumatic neuronal loss and neurological deficits. TBI-induced cell cycle activation (CCA) in post-mitotic neurons causes regulated cell death involving cyclin-dependent kinase (CDK) activation and initiation of an E2F transcription factor-mediated pro-apoptotic program. Here we examine the mechanisms of CCA-dependent neuronal apoptosis in primary neurons in vitro and in mice exposed to controlled cortical impact (CCI). In contrast to our prior work demonstrating neuroprotective effects by CDK inhibitors after TBI, examination of neuronal apoptotic mechanisms in E2F1-/-/E2F2-/- or E2F2-/- transgenic mice following CCI suggests that E2F1 and/or E2F2 likely play only a modest role in neuronal cell loss after brain trauma. To elucidate more critical CCA molecular pathways involved in post-traumatic neuronal cell death, we investigated the neuroprotective effects and mechanisms of the potent CDK inhibitor CR8 in a DNA damage model of cell death in primary cortical neurons. CR8 treatment significantly reduced caspase activation and cleavage of caspase substrates, attenuating neuronal cell death. CR8 neuroprotective effects appeared to reflect inhibition of multiple pathways converging on the mitochondrion, including injury-induced elevation of pro-apoptotic Bcl-2 homology region 3 (BH3)-only proteins Puma and Noxa, thereby attenuating mitochondrial permeabilization and release of cytochrome c and AIF, with reduction of both caspase-dependent and -independent apoptosis. CR8 administration also limited injury-induced deficits in mitochondrial respiration. These neuroprotective effects may be explained by CR8-mediated inhibition of key upstream injury responses, including attenuation of c-Jun phosphorylation/activation as well as inhibition of p53 transactivation of BH3-only targets.

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Poster Presentation 239

Abstract Title:	**Phenelzine, Pargyline, and Hydralazine: The effects of Lipid Peroxidation-Derived Aldehyde Scavenging and Monoamine Oxidase Inhibition on Learning and Memory and Cortical Tissue Sparing Following Experimental TBI**
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Abstract: In the US, over 5 million people suffer from a traumatic brain injury (TBI)-related disability. There are no neuroprotective FDA-approved pharmacotherapies for TBI. Lipid peroxidation-derived neurotoxic aldehydes contribute to neuronal death and neurologic dysfunction after TBI. Phenelzine (PZ) contains a hydrazine moiety capable of scavenging aldehydes. Therefore, PZ can improve mitochondrial bioenergetics and reduce aldehyde load following experimental TBI. However, PZ is an FDA-approved monoamine oxidase inhibitor (MAOI)-class anti-depressant and the effect MAO inhibition has on TBI is unknown. The goal of this study was to compare the ability of PZ (aldehyde scavenger, MAOI), hydralazine (HZ, aldehyde scavenger, non-MAOI) and pargyline (PG, non-aldehyde scavenger, MAOI) to improve learning and memory and cortical tissue sparing following severe CCI in 3mo male Sprague-Dawley rats. PZ (15mg/kg), HZ (5mg/kg), PG (15mg/kg), or vehicle (saline) were administered intraperitoneal 15min, 24h, and 48h post-CCI. Morris Water Maze (MWM) was conducted post-injury D3-7. Animals were euthanized and perfused post-CCI D8. The same dosing paradigm was utilized in uninjured animals and cortical tissue was sent for HPLC analysis of monoamines and their metabolites. The results indicate that neither PZ, HZ, nor PG improved CCI-induced deficits to retention memory or cortical tissue sparing. However, HZ performed the best, improving cortical tissue sparing compared to vehicle by 10%. Concerningly, PZ was the only group to not show significant improvement during the MWM acquisition phase, and lost significantly more weight than all other groups, possibly due to an increase in norepinephrine or serotonin as was seen in uninjured cortical tissue.

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