



Welcome to the 2020 Creighton Neuroscience Symposium!

Hosted by the CUSOM Department of Pharmacology & Neuroscience

Date: Friday, Sept 11, 2020

Organizers: Drs. Holly Stessman and Shashank Dravid

Schedule of events

8 AM – 5 PM

Virtual presentations from CU trainees open for browsing and discussion board Q & A

“Live” Zoom events:

12 – 1 PM

Lunchtime Panel Discussion (*Topic: Overcoming hurdles in translating research findings to the bedside*)

3 – 4 PM

Breakout sessions

- (1) CU undergraduate neuroscience opportunities and the 4+1 Neuroscience Master's program
- (2) Integrating translational research into clinical practice
- (3) Post-PhD: Taking your next step

ABSTRACTS

Theme: EPILEPSY AND SEIZURES

Presenter: Ross Johnson, Medical student

Mentor: Dr. Susan Herman

Institution: Barrow Neurological Institute Department of Neurology

Background: The Epilepsy Learning Healthcare System (ELHS) aims to improve outcomes for epilepsy patients through continuous quality improvement. ELHS Patient Reported Outcomes (PROs) measure quality of life, medication adherence, and mental health. **Methods:** Plan-Do-Study-Act cycles were performed at Barrow Neurological Institute to assess the feasibility of pre-clinic telephone and electronic PROs collection. In Cycle 1, epilepsy patients were contacted by phone and completed a survey including demographics and PROs. In Cycle 2, surveys were emailed to patients; if no response, patients were called to complete surveys by phone. Survey data was made available to providers prior to the clinic visit. **Results:** Cycle 1: 9/16 (56%) of patients were contacted by phone; 7/16 (43%) completed the interview, taking 20-30 minutes per interview. Cycle 2: 2/22 patients completed survey by email; 6/8 were completed by phone; total completion rate 8/22 (36%). **Conclusion:** Collection of pre-visit PROs by phone and email had low completion rates and high time burden. PDSA quality improvement methodology can be used to rapidly assess feasibility and workflow of practice innovations. Provider pre-visit review of PROs promoted more efficient visits focusing on medical issues most important to the patient. Future PDSA cycles will aim to improve PRO completion rates.

Presenters: Cameron Booth and Shelby Herr, Medical students

Mentor: Kristina Simeone

Institution: CUSOM - Pharmacology and Neuroscience

Autoresuscitation is the body's way of "waking itself up" when the brain becomes hypoxic. More specifically, when the body is deprived of oxygen, it goes through a cycle of rapid ventilation, bradycardia and gasping in order to return to normalcy. We believe that failure of autoresuscitation could be a cause of Sudden Death in Epilepsy (SUDEP). We induced the cycle of autoresuscitation in epileptic *Kcna 1^{-/-}* mice by exposing them to a challenge of hypercapnia and hypoxia (HH), and we examined their breathing patterns before, during, and after the challenge. This data was compared to that of a sacrificed control mouse of the same age. As KO mice were subjected to successive rounds of respiratory challenges, their time spent inspiring increased at the expense of time spent expiring, represented as a continued increase in duty cycle (T_i/T_{total}). In contrast, the duty cycle of WT mice did not change between each subsequent challenge, suggesting that KO mice gradually become desensitized to the

accumulating CO₂ over time while WT mice do not. Applying this to epileptic patients, respiratory parameters such as duty cycle and expiration duration could be used as markers for SUDEP risk, allowing for its prediction and prevention.

Presenter: Thomas Gossard, Medical student

Mentor: Dr. Sanjay Singh

Institution: CUSOM - Neurology

There are over 20 different anti-epileptic drugs (AED) that may be used in the treatment of epilepsy; however, there is a relative paucity of data regarding overall treatment outcomes of epilepsy specific to women. Our study goal was to evaluate first-line epilepsy therapy and outcomes in an entirely female cohort. Data was gathered retrospectively from women aged 19-89 who had been diagnosed with epilepsy for the first time between 2015 and 2019 at the CHI Health-Creighton University Epilepsy Center. Sixty-two women were identified via retrospective chart review. Patients were deemed responsive to therapy if their seizure frequency was reduced by 50%. The most commonly prescribed first line medication was levetiracetam (n=55), to which 70% (n=39) responded to treatment, 11% (n=6) did not respond, and 18% (n=10) were lost to follow up. Four patients were prescribed lamotrigine as first-line therapy; two responded to AED therapy and two were lost to follow up. Lacosamide was the third most commonly used (n=2), with one patient responding and one exhibiting refractory epilepsy. One patient refused AED therapy, but did not have any future documented seizures. Overall, our findings suggest that anti-epileptic treatment for women at this center aligns with the expert guidelines for AEDs.

Presenter: Joseph A Kostansek IV, Graduate student

Mentor: Dr. Kristina Simeone

Institution: CUSOM - Pharmacology and Neuroscience

The hippocampus contains orexin receptors and receives projections from orexin neurons in the lateral hypothalamus. Orexin excites neurons, induces long-term potentiation, and at high doses, causes seizures. In the *Kcna1*-null mouse model of epilepsy, there is an increased number of orexin positive neurons and blocking orexin receptors reduces seizures. We will determine (i) whether orexin contributes to synaptic plasticity and spontaneous activity in control slices and (ii) whether the role of orexin differs between wild-type and epileptic tissue. Horizontal mouse hippocampal slices from wild-type and *Kcna1*-null mice were placed over a 64-electrode grid. Slices were treated with control artificial cerebral spinal fluid (aCSF) or aCSF containing 100nM TCS-1102 – a dual orexin receptor antagonist. Electrodes at Schaffer Collateral-CA1 synapses delivered paired-pulse stimulations to evoke field potentials. These electrodes also recorded spontaneous activity in the form of sharp waves and high-frequency oscillations (SPW-HFOs) before, during, and after TCS-1102 treatment. In wild-type and knockout slices, TCS-1102 changed SPW characteristics. TCS-1102 has differing effects on

HFOs in wild-type and knockout slices. TCS-1102 influenced the paired-pulse ratio in both slice types. These data indicate that endogenous orexin may play a role in modulating hippocampal spontaneous activity. Further data generated from this study will determine the role of orexin in the hippocampus and will also generate insight as to how the orexin system can be targeted to treat epilepsy.

Theme: NEUROBIOLOGY OF HEARING

Presenter: Joe DiGuiseppi, Medical student

Mentor: Dr. Sarath Vijayakumar

Institution: CUSOM – Biomedical Sciences

Noise-induced hearing loss (NIHL) is a common type of hearing loss that affects many individuals who work in loud environments such as industrial and agricultural workers, military, construction workers, and professional musicians. The exact molecular mechanism of NIHL is uncertain, and there are currently no FDA-approved drugs to treat NIHL. Since there is no in vitro assay that can accurately replicate the inner ear environment during noise exposure, we used an in silico approach to determine which biological pathways and drug targets are of most interest to the prevention and treatment of NIHL. Differentially expressed genes from published noise-treated mouse cochlear transcriptomes were input to the NIH Library of Integrated Network-based Cellular Signatures (LINCS) L1000CDS2 program and ShinyGO enrichment pathway analysis program, and a list of 183 drug perturbations that mimic or reverse the input transcriptome was provided. Based on five comparisons from two published NIHL transcriptomes, 51 drug candidates and 18 biological pathways were identified for the prevention and/or treatment of NIHL based on DNA microarray and RNA-seq transcriptomic analysis. 11 of the 51 drug candidates are FDA-approved and will be prioritized for in vivo testing and repurposing as an NIHL treatment in the future.

Presenter: Joseph Frank, Undergraduate student

Mentor: Dr. Jian Zuo

Institution: CUSOM – Biomedical Sciences

Atoh1 and Pou4f3 are two genes well-known for their involvement in mammalian hair cell development. Either Atoh1 overexpression alone or Pou4f3 overexpression alone can support the direct transdifferentiation of nonsensory supporting cells into immature hair cells (HCs). Our previous work has shown that in adult mouse cochleae, Atoh1 overexpression alone results in ~5 new HCs per cochlea, Pou4f3 overexpression alone results in ~25 new HCs per cochlea, and that combining Atoh1 and Pou4f3 overexpression vastly enhances the conversion rate, resulting in ~160 new HCs per cochlea. To identify small molecules that can promote HC

regeneration, we screened over 45,000 compounds for POU4F3 activation in our human POU4F3 promoter-driven dual-luciferase cell line. From this screen, we found 86 small molecules that significantly increase POU4F3 transcriptional activity, 12 of which are known for their mechanisms of action. Our top compounds significantly increase cochlear Pou4f3 mRNA levels when delivered transtympanically in adult mice. Therefore, we have identified small molecule Pou4f3 agonists that may be therapeutically beneficial for cochlear hair cell regeneration in adult mammals.

Presenter: Madeleine Urbanek, Undergraduate student

Mentor: Dr. Jian Zuo

Institution: CUSOM – Biomedical Sciences

Tinnitus is a highly prevalent yet distressing condition marked by the perception of phantom sounds within the ear. While tinnitus affects more than 10% of the world's population, there are currently no effective treatment options for those patients with particularly debilitating cases. Though awareness of the genetic predisposition responsible for the development of tinnitus may prove useful for halting its onset, studies of its underlying genetic foundation have failed to reveal any definitive conclusions. Taking a genome-wide association approach, we used the UK Biobank's collection of 50,000 exome sequences to compare the genetic profiles of individuals having never reported tinnitus to individuals reporting chronic tinnitus, while controlling for other confounding health conditions. Our project, with a larger cohort size than any genetic tinnitus study published to-date, will assist in identifying any tinnitus-linked single nucleotide polymorphisms and their corresponding gene's contribution to the development of tinnitus. Supported by the Clare Boothe Luce Scholarship and LB692. Acknowledgement to Dr. Richard Tyler (Univ. Iowa) for his advice.

Presenter: Jonathan Fleegel, Graduate student

Mentor: Dr. Jian Zuo

Institution: CUSOM – Biomedical Sciences

Zebrafish are a well-established model organism in the field of auditory research. A special feature of Zebrafish that make it a valuable model is the presence of mechanosensory hair cells on the lateral lines of these fish. These hair cells are homologous to the hair cells present in the inner ear and their superficial location allows for easy assessment and drug treatment through direct administration to the fish water. In addition, their small size, transparent tissue, and rapid development make them an ideal organism to do high throughput drug ototoxicity screening in vivo. In my own research endeavors, I have utilized this organism in studying aminoglycoside related hair cell toxicity. Our goal is to use this model organism to identify otoprotective compounds and identify the mechanisms by which these compounds confer protection. Specifically, we will utilize the strengths of this model organism and pair it with powerful genetic

manipulation tactics such as morpholino oligonucleotide gene knockdown to isolate and characterize the primary targets of otoprotective compounds. Overall, this model organism has provided an efficient and practical screening method for the identification of potential otoprotective drugs and their mechanisms.

Theme: NEUORPATHOGENS

Presenter: Luke Kiefer, Medical student

Mentor: Dr. Melinda Burnett

Institution: CUSOM

Intracranial hypertension secondary to viral infection (IHSVI) is a rare presentation of viral meningitis demonstrating elevated cerebrospinal fluid (CSF) pressure (>25 cm H₂O), characteristic CSF pleocytosis, and unremarkable radiographic imaging. Commonly described IHSVI etiologies include HIV, Measles, and Varicella Zoster Virus (VZV). Similar to idiopathic intracranial hypertension (pseudotumor cerebri), patients experience headache, nausea, papilledema, diplopia, and visual field restrictions. In a recent case report, our team identified two immunocompetent middle-aged individuals with IHSVI. CSF polymerase chain reaction (PCR) testing confirmed VZV and Human Herpesvirus 6 (HHV-6) infections in our male and female patients respectively. The VZV patient responded positively and rapidly to acetazolamide and valacyclovir, transitioning to ongoing acetazolamide monotherapy. The HHV-6 patient, who had a more robust viral infection measured by PCR, required several weeks of acetazolamide and ganciclovir therapy to achieve symptom remission and remains on acetazolamide monotherapy as well. Because of the powerful role of specific therapeutics in IHSVI, diagnostic vigilance is warranted in patients presenting with new-onset, persistent headache. Therefore, we recommend investigators not only measure lumbar puncture opening pressure, but also conduct basic CSF studies and viral PCR analysis on spinal fluid in all patients with suspected intracranial hypertension.

Presenter: Alyssa Block, Graduate student

Mentor: Dr. Jason Bartz

Institution: CUSOM - Medical Microbiology and Immunology

Prions are comprised of PrP^{Sc}, the misfolded isomer of the cellular protein PrP^C. Prions are zoonotic and the mechanisms that govern interspecies transmission are unknown, resulting in an inability to predict the zoonotic potential of emerging prion diseases. In vitro generation of prions from minimal components, (i.e. synthetic prions), have provided insight into the mechanisms of PrP^{Sc} formation. It is unknown if synthetic prions can infect a different species, knowledge necessary to further understand mechanisms behind interspecies transmission. To

investigate this possibility, Syrian hamsters were inoculated with murine synthetic prions that resulted in all of the inoculated hamsters developing clinical signs of prion disease. Serial intraspecies transmission resulted in shortening and stabilization of the incubation period consistent with prion adaptation. The passage history, clinical signs and biochemical properties of PrPSc from these animals is consistent with the hypothesis that inoculation of hamsters with murine synthetic prions resulted in the reisolation of 139H, a hamster prion strain isolated from mouse prions. From these observations, we conclude that synthetic prions recapitulate a known interspecies transmission event with native brain derived prions. Therefore, synthetic prions can be utilized to study the mechanisms of interspecies transmission in a simplified trackable system.

Presenter: Tess Gunnels, Graduate student

Mentor: Dr. Jason Bartz

Institution: CUSOM - Medical Microbiology and Immunology

Prions are comprised of PrPSc, the misfolded isoform of the host encoded protein PrPC, and are the causative infectious agent of inevitably fatal neurodegenerative diseases in humans and other mammals. Prion strains are operationally defined as a heritable phenotype of disease that can differ in its clinical signs, incubation period, tissue tropism, and host range. These strain-specific properties are thought to be encoded by strain-specific conformations of PrPSc. There is evidence that multiple prion strains propagate in a subset of individuals with natural prion diseases, suggesting that prions exist as a mixture of strains, however direct evidence for this hypothesis is lacking. Using a novel biochemical technique termed conformational stability and selection assay (CSSA), we can selectively eliminate PrPSc from a hamster-adapted prion strain called DY, but not affect PrPSc from other hamster-adapted prion strains. When we apply the CSA to DY prions that are homogeneous by all biological criteria, we would predict complete inactivation of DY, but instead we have identified emergence of non-DY strains. These data provide the first direct evidence that prion strains exist as a mixture which has a number of implications for developing prion therapeutics and prion host range.

Presenter: Alexandria Jones, Undergraduate student

Mentor: Dr. Amy Worthington

Institution: CU – Biology, Neuroscience

Parasites have evolved impressive mechanisms of manipulating their host to maximize their fitness. Using male sand field crickets, *Gryllus firmus*, I will identify the major behavioral and neural changes of crickets infected with the long-lived parasitic horsehair worm, *Paragordius varius*, at critical timepoints in this host-parasite interaction. During early developmental stages, the cricket host and horsehair worm have common interests of survival, resource acquisition, and growth. Upon cricket maturity, however, crickets shift their behaviors to maximize

reproductive fitness, subsequently putting the parasite's survival at risk. The parasite manipulates its host to minimize such behaviors, and subsequently manipulates host behavior again when the parasite is ready to emerge as a reproductive adult in an optimal emergence environment. I will perform behavioral assays (courtship calling, aggression, locomotion, and water-seeking behavior) to analyze the host manipulation by the parasite, and additionally analyze neurotransmitter levels in the host cricket's brain at these two critical time points in this host-parasite interaction. By linking the modified behaviors of infected cricket to manipulated levels of serotonin and octopamine, I will explore the causal neurological mechanisms leading to host behavioral manipulation during critical time points of an intricate host-parasite interaction.

Theme: NEUROIMMUNE SYSTEMS

Presenter: Olivia Burleigh, Undergraduate student

Mentor: Dr. Annemarie Shibata

Institution: CU – Biology, Neuroscience

Activated microglia function in the central nervous system as immune responsive cells. Certain long non-coding RNAs (lncRNAs) may be important regulators of the pro-inflammatory state of microglia. lncRNAs are functional RNAs that do not encode proteins but help facilitate gene regulation. Our hypothesis is that upon activation by pro-inflammatory stimuli, microglia differentially express a set of lncRNAs that enhance the immune response. We used a genome-wide microarray analysis of LPS stimulated microglia compared to control microglia to screen for differentially expressed lncRNAs. Upon analysis, we identified several upregulated lncRNAs and validated the results using RT-qPCR. We stimulated the BV2 murine microglia cell line and primary microglia with LPS and observed a significant increase in a novel lncRNA, lncRNA-25B, and positive controls. Temporal expression was analyzed utilizing a time course experiment in response to stimuli such as LPS, TNF- α , and lfn- γ , and demonstrated that lncRNA-25B appears to act early in the inflammatory response, with expression returning to baseline by 24h. Additionally, infection of BV2s with TMEV, a murine virus used to model multiple sclerosis, demonstrated a significant increase in expression of lncRNA-25B. Identification of lncRNAs that modulate the inflammatory response could provide a novel target for pharmaceutical therapeutics.

Presenter: Nicholas Mathy, Medical student

Mentor: Dr. Annemarie Shibata

Institution: CU – Biology, Neuroscience

Long non-coding RNAs (lncRNAs) are transcripts which lack protein coding potential, but can regulate gene expression via interactions with RNA-binding proteins. Previous work has shown

that one lncRNA, referred to as lncRNA-25B, is induced in murine microglia in response to inflammatory stimuli. Knockdown of lncRNA-25B using siRNA prior to LPS stimulation resulted in a significant decrease in the expression of iNOS when compared to scrambled siRNA. Conversely, overexpression of lncRNA-25B prior to stimulation resulted in a significant increase in iNOS when compared to an empty vector control. We probed for an interaction between lncRNA-25B and NF- κ B p65 by using RNA immunoprecipitation, as iNOS is an NF- κ B target gene. After LPS stimulation, there was a significant increase in the interaction between NF- κ B p65 and lncRNA-25B compared to control IgG, and compared to the unstimulated condition. To test whether lncRNA-25B enhances NF- κ B p65 binding to the promoter region of iNOS, chromatin immunoprecipitation was used. Knockdown of lncRNA-25B significantly reduced the enrichment of NF- κ B p65 to the iNOS promoter at two distinct sites when compared to the scrambled siRNA control. Together, these results suggest lncRNA-25B is involved in the transcriptional regulation of iNOS. Future directions will investigate the role of lncRNA-25B *in vivo*.

Presenter: Rachael Urquhart, Graduate student

Mentor: Jee Yeon Hwang; Dr. Gopal Jadhav

Institution: CUSOM - Pharmacology & Neuroscience

Global ischemic stroke results from impaired cerebral blood flow, usually following cardiac arrest. During global ischemia the entire brain is deprived of oxygen. The hippocampus is most strongly affected, showing selective delayed death of CA1 neurons. Ischemia triggers an inflammatory response to address neuronal damage. Emerging evidence reveals neuroinflammation is an important element in ischemic stroke; however, the underlying mechanisms and potential therapeutic strategies must be further elucidated. In this study, our RNA-seq and bioinformatic analysis reveals that immune response related pathways including TREM1 (Triggering receptor expressed in myeloid cells 1) signaling and neuroinflammation are the top canonical pathways in global ischemia-induced neuronal death in hippocampal CA1 in rats. TREM1 is an inflammatory type I membrane receptor expressed in myeloid lineage and magnifies the proinflammatory innate immune response. Here we show TREM1 and its regulatory partner DAP12 are activated at 48h post-ischemia *in vivo* and TREM1 inhibition by our newly synthesized antagonists affords neuroprotection in primary hippocampal neurons subjected to oxygen glucose deprivation, an *in vitro* model of ischemia. These findings suggest that TREM1-mediated neuroinflammation is causally related to neuronal death and identify TREM1 as a potential therapeutic target for amelioration of neurodegeneration associated with global ischemia.

Theme: BIOLOGY OF RARE GENETIC DISORDERS

Presenter: Carly Baker, Undergraduate student

Mentor: Dr. Annemarie Shibata

Institution: CU – Biology, Neuroscience

Carnitine palmitoyltransferase II (CPTII) facilitates conversion of palmitoylcarnitine to palmitoyl-CoA. CPTII deficiency is associated with neurological deficits and may play a role in neuropsychological disorders such as epilepsy, attention deficit disorder, intellectual disabilities, autism, and schizophrenia. The purpose of this project is to understand how CPTII influences formation and function of the vertebrate nervous system. Zebrafish are used in this project as the vertebrate model organism since they are quick to mature and show similar processes of nervous system development and function to humans. Microinjection of translation blocking and splice blocking morpholinos directed against CPTII into fertilized zebrafish embryos knocked down CPTII message and protein expression. Scrambled morpholino injected zebrafish were used as controls. Phenotypes of CPTII knockdown conditions were analyzed at 2 days (hatching stage) and 5 days (larval stage) post-fertilization and compared to controls. CPTII-deficient zebrafish developed short, curved tails, abnormal pigmentation, abnormal eye shape, and decreased distance between eyes. Alcian blue staining showed abnormal facial cartilage in CPTII-deficient zebrafish. Oil Red staining revealed that CPTII-deficient zebrafish develop abnormal blood vessels and have increased lipid deposits. Continued histological and behavioural analyses are underway to better characterize the role of CPTII in brain development, function, and disorders.

Presenter: Michelle Ngo, Medical student

Mentor: Dr. Zach, Dr. Pedersen

Institution: CUSOM – Neonatology

Septo-optic dysplasia (SOD), sometimes known as De Morsier Syndrome, is a rare congenital condition classically characterized by the triad of optic nerve hypoplasia, midline cortical defects, and hypothalamic-pituitary dysfunction, though manifestations vary greatly in severity, clinical presentation, and phenotype. There have been a variety of endocrine manifestations, such as growth hormone deficiency. There have also been a wide range of other ocular and brain abnormalities reported in SOD patients, such as corpus callosum dysgenesis, schizencephaly, and olfactory tract hypoplasia. When cortical dysplasia is present, the disorder is termed SOD-plus. The cause of this irregular early brain development is not completely understood, but most cases seem to be sporadic while some familial cases point to mutations in developmental genes such as HESX1, SOX2, or SOX3. This case report describes a patient with polymicrogyria, septum pellucidum agenesis, and probable Rathke's cleft cyst (RCC) on imaging. SOD was considered in our patient, but based on the extreme variability of SOD, we suggest that the classical triad of symptoms should not be considered definitive requirements for diagnosis. Physicians should continue to keep suspected cases of SOD in their differentials even if their patients do not meet two of the three common characteristics.

Presenter: Jason Hulen, Graduate student

Mentor: Dr. Holly A.F. Stessman

Institution: CUSOM – Pharmacology and Neuroscience

KMT5B is a histone methyltransferase identified as an autism spectrum disorders risk gene with observed symptoms that match a subgroup with motor development delay symptoms. KMT5B mRNA is expressed in tissues related to movement: motor cortex, motor nerves, and skeletal muscle. Fragmentation at the neuromuscular junction, the interface between brain and muscle, can occur in myopathies and neuropathies to produce motor deficits. We hypothesized that there are structural changes at the neuromuscular junction due to loss of Kmt5b. Twenty-four wild-type and 15 heterozygous Kmt5b mice's hindlimb muscles (soleus and extensor digitorum longus) were dissected and immunofluorescence of whole-mount muscle tissues with confocal microscopy was performed to quantify area and discontinuity of the neuromuscular junction. Each parameter was compared using independent samples t-test. In soleus muscle and extensor digitorum long muscle, discontinuity is increased in the heterozygous Kmt5b mouse (mean difference: 2.71, 95% CI: 0.35 – 5.07, $p = 0.025$, $d = 0.76$ and mean difference: 2.33, 95% CI: -0.021 – 4.68, $p = 0.052$, $d = 0.69$, respectively). Our results indicate an increase in fragmentation of the neuromuscular junction in Kmt5b heterozygous mice. This suggests that structural differences may contribute to motor abnormalities and developmental delays in individuals carrying disruptive KMT5B variation.

Theme: BASIC NEUROSCIENCE AND MODEL SYSTEMS

Presenter: Segewkal Hawaze Heruye, Graduate student

Mentor: Dr. Timothy A. Simeone

Institution: CUSOM – Pharmacology and Neuroscience

The dose-dependent effect of ascorbic acid (AA) in impairing short-term and long-term memory is reported in *in vivo* studies. However, the effects of AA on neuronal transmission and different forms of plasticity are not yet understood. Here, we determined the concentration-dependent effect of AA (200 μM , 400 μM , 1mM, and 2 mM) on three forms of plasticity at the CA3-CA1 synapse: paired-pulse facilitation (PPF), short-term potentiation (STP) and early long-term potentiation (eLTP) which are molecular correlates of memory *in vitro*. All four concentrations of AA reduced baseline synaptic activity (field excitatory postsynaptic potential and synaptic strength). 200 μM , 400 μM , and 2mM AA reduced STP while all concentrations of AA reduced eLTP. None of the AA concentrations affected paired-pulse ratio after LTP induction, which is inversely related to the probability of neurotransmitter release. This study demonstrates that AA has a concentration-dependent effect on various forms of plasticity. The impaired plasticity observed with the lower and higher concentrations of AA could be attributed to the pro-oxidant property of AA at sub-optimal and supra-optimal concentrations. These changes in molecular correlates of memory could be mechanisms for short-term and long-term memory impairments observed with a low dose of AA *in vivo*.

Presenter: Mary Elizabeth Yeh, Medical student

Mentor: Dr. Timothy A. Simeone

Institution: CUSOM – Pharmacology and Neuroscience

100 million Americans are affected by one or more neurological diseases. Initial pharmacotherapy development relies heavily on in vitro or in vivo studies—with the latter entailing significant financial, labor, and time investment. However, there exist in vitro culture systems that bypass the investments and restrictions inherent to animal research. We aim to characterize one novel in vitro culture system that reduces these costs. NTera2/cl.D1 (NT2) cells, a clonally-derived pluripotent human embryonal carcinoma cell line, differentiate into functional neurons after 8-week treatment with retinoic acid (4 weeks) then mitotic inhibitors (4 weeks). However, based on work by González-Burguera et al. (2016), we found that 6-day treatment with 20 μ M of the nucleoside analogue cytosine-B-D-arabinofuranoside yielded differentiated neurons and astrocytes that remained viable for >12 days post-differentiation. Immunofluorescence showed that the NT2 neurons and astrocytes produced using this novel method existed in a 2:1 ratio. Furthermore, glutamatergic and GABAergic subtypes existed in a 4.8:1 ratio (similar to the human brain in vivo). Preliminary electrophysiological studies indicate that these cells possess the machinery for spontaneous action potentials. Developing this in vitro system of accelerated neuronal network establishment will decrease drug screening costs and potentially reduce the use of animals in preclinical studies.

Theme: ENVIRONMENT X NEUROBIOLOGY INTERACTIONS

Presenter: Marley Bredehoeft, Graduate student

Mentor: Dr. Janee Gelineua-van Waes and Dr. Holly Stessman

Institution: CUSOM - Pharmacology and Neuroscience

Surveillance studies in Botswana show increased prevalence of neural tube defects (NTDs) in babies born to women taking the antiretroviral dolutegravir (DTG) prior to conception. DTG acts by chelating magnesium (Mg²⁺) in the active site of the HIV integrase. Magnesium has established importance in pregnancy maintenance. Mouse models were used to test the hypothesis that decreased Mg²⁺ availability from DTG chelation could affect embryonic development, especially with compounding gene-nutrient interactions that compromise Mg²⁺ homeostasis. 2 mouse strains with different basal levels of plasma Mg²⁺ were selected. Weanlings were placed on diets containing sufficient or high Mg²⁺. Mice were placed in metabolic cages for 3 days and urine samples collected. Treatment on pregnant females began on E0.5 and embryos harvested/phenotyped on E9.5. On high Mg²⁺ diet, no NTDs were observed in either strain treated with vehicle or low dose DTG; however, on high dose DTG, exencephalic embryos were observed in the strain with lower plasma Mg²⁺. Exencephaly was also observed in the 'susceptible' strain when dams on Mg²⁺ sufficient diet were treated with

low dose DTG. In addition, whole genome sequencing data were used to call relevant magnesium homeostasis genes as potential 'susceptibility' candidates. This study presents the first animal model of DTG-induced NTDs that can be used to study underlying mechanism(s), and genetic and nutritional risk factors that contribute to susceptibility.

Presenter: Ally Einbeck, Undergraduate student

Mentor: Dr. Charles Bockman

Institution: CUSOM - Pharmacology and Neuroscience

Electronic cigarettes or e-cigarettes containing nicotine are abused for their rewarding effects and are used as a nicotine replacement therapy to help tobacco smokers quit. Due to their novelty, the long-term effects of e-cigarette use are unclear. Thus, this study examined the long-term effects of e-cigarettes on mice airways. E-cigarettes deliver nicotine in an aerosol known as "vaping". Vaping uses an "e-liquid" containing nicotine, propylene glycol (PG) and vegetable glycerin (VG). PG/VG serves as the vehicle transporting nicotine. Preliminary studies indicate e-cigarette aerosol produces inflammation-dependent bronchial hyper-contraction. Airway inflammation results in smooth muscle hyperplasia, explaining increased contractility. Nicotine, VG and PG can cause inflammation. Thus, our objective was to determine whether nicotine or vehicle causes altered airway smooth muscle. Airway smooth muscle will be quantified in 5-micron sections stained for actin. Three groups of mice are currently being exposed to air, e-cigarette aerosol containing nicotine or vehicle-only aerosol for 10-weeks. My hypothesis is e-cigarette aerosol produces airway smooth muscle hyperplasia caused by PG and VG independently of nicotine. Structural change in airways would result in our reported increase in bronchial contractility. These data would suggest e-cigarette-induced changes in airway smooth muscle causing bronchial hyper-contraction would increase risk for bronchospasms.

Presenter: Marika Marklin, Undergraduate student

Mentor: Dr. Amy Badura-Brack

Institution: CU - Psychology

MANCOVA revealed that ventral DMN volume was significantly larger in children with high versus low trait dissociation after controlling for trauma. Follow-up ANCOVAs found the strongest differences in the precuneus. Trait dissociation includes cognitive disruptions in memory and self-awareness, which are functions of the precuneus and risk factors for psychopathology.