PLATFORM TALK

Enteric nervous system macrophages exhibit functional decline and an age-dependent phenotype similar to disease-activated microglia

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Muscularis macrophages (MMs) are a tissue-resident population of macrophages associated with the enteric nervous system (ENS) of the gastrointestinal (GI) tract. MMs typically express an anti-inflammatory phenotype and support ENS homeostasis. In aging, the MM phenotype shifts to a pro-inflammatory state that is associated with GI dysmotility. In Alzheimer's disease, microglia develop a unique inflammatory signature called the disease-activated macrophage (DAM) phenotype. Here, we hypothesize that muscularis macrophages (MMs) in aged mice develop a pro-inflammatory state that is similar to the DAM phenotype observed in neurodegeneration.

To test this, we isolated immune cells from the small intestine, colon, and spinal cord of young (3 months) and aged (16-24 mxonths) WT C57BL/6 mice. Cells were dissociated and sorted by fluorescent-activated cell sorting (FACS) to facilitate gene analysis by quantitative real-time PCR (qPCR) and single-cell RNAseq. Young and aged mice each exhibited a population of homeostatic MMs characterized by microglial gene expression *(P2ry12, Trem2, Gpr34).* A population of geriatric state (GS) MMs was identified in aged mice that express DAM genes *(CD9, Itgax, Clex7A).* GS cells exhibit reduced phagocytosis of pHrodo beads and reduced clearance of α-synuclein.

To confirm the clinical translation of these findings, we dissociated cells from human colon samples and sorted them by FACS to facilitate gene analysis by qPCR. We identified a population of human MMs (MM_1) consistent with those observed in mice that exhibits homeostatic microglial genes. In aging, the human MM_1 cells develop a GS phenotype that is preferentially associated with α-synuclein accumulation.

To summarize, mice and human MMs exhibit a homeostatic phenotype similar to microglia. With aging, MMs develop a pro-inflammatory GS phenotype similar to the DAM microglia observed in neurodegenerative disease. This shift in phenotype likely drives changes in enteric neuron survival and decreased gastrointestinal motility previously observed in aging.

Left- vs right-sided headache in migraine

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Migraine classically involves unilateral headache. In fact, the word migraine is derived from the Greek *hēmikrania* or half skull. And unilateral headache remains a criterion which is supportive of the clinical diagnosis of migraine of the International Classification of Headache Disorders.

Why headache is often unilateral and whether the side in which headache occurs matters is not currently known. In a series of studies, we have begun to examine how migraine with left- vs right-sided headache may differ.

In the first study, a scoping review, we examined what is currently known about migraine with left- vs right-sided headache. Left/right migraine were found to differ across multiple domains, including psychiatric comorbidities, performance on cognitive testing, autonomic testing, and imaging.

In the second study, patients who experienced “typically” left- or right-sided headache during attacks were identified by reviewing the clinical intake questionnaires of all patients diagnosed with migraine at the UVM Headache Clinic over the past 20 years. Patients with left-migraine were found to have more days per month and more severe headaches days per month than those with right-migraine.

In the third study, using the same cohort, for each patient that had a clinical brain MRI available in the UVM picture archiving and communication system, white matter hyperintensities were counted and their location documented by a team of radiology residents blinded to headache laterality. Patients with left-migraine had >50% more white matter hyperintensities than those with right-migraine, after adjusting for multiple cardiovascular risk factors.

In a fourth study, participants with left/right migraine underwent functional MRI during a spontaneous unilateral attack, while head pain was at least moderately severe, prior to using acute pain relievers. Participants then returned on a separate day and repeated the scan when they were free of all migraine symptoms. No differences in vascular reactivity during vs between attacks were identified in participants with left-migraine. However, multiple brain regions showed alterations in vascular reactivity in participants with right-migraine.

Early life exposure to broccoli sprouts confers stronger protection against enterocolitis development in an immunological mouse model of inflammatory bowel disease

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To our knowledge, IL-10-KO mice have not previously been used to investigate the interactions of host, microbiota, and broccoli, broccoli sprouts, or broccoli bioactives in resolving symptoms of CD. We showed that a diet containing 10% raw broccoli sprouts increased the plasma concentration of the anti-inflammatory compound sulforaphane and protected mice to varying degrees against disease symptoms, including weight loss or stagnation, fecal blood, and diarrhea. Younger mice responded more strongly to the diet, further reducing symptoms, as well as increased gut bacterial richness, increased bacterial community similarity to each other, and more location-specific communities than older mice on the diet intervention. Crohn's disease disrupts the lives of patients and requires people to alter dietary and lifestyle habits to manage symptoms. The current medical treatment is expensive with significant side effects, and a dietary intervention represents an affordable, accessible, and simple strategy to reduce the burden of symptoms.

Bridging two worlds: A story of motility and behavior

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OBJECTIVE: There is a high degree of comorbidity between gastrointestinal diseases and psychopathologies, underscoring the need for a better understanding of the bidirectional communication between the gut and the brain. The gut microbiota is an important player in a wide array of functions, ranging from intestinal health to brain neurochemistry and behavior. Exactly how the effects of the gut microbiota are mediated in the brain remain to be elucidated. Serotonin, made from the amino acid tryptophan (Trp), is a key neurotransmitter which can regulate behavior. Here we bridge our previous findings that bacterial production of Trp can increase 5-HT production in the gut and ask whether there is a similar response in the brain.

METHODS: *Bacillus* (*B.) subtilis,* is known to express Trp synthase and for its ability to generate Trp. Male C57BL6 mice received an oral gavage of *B. subtilis R0179* (10^9 CFUs daily) or vehicle (PBS) for one week. On day 7, blood samples and tissues from the brainstem and hippocampus were collected. Levels of Trp, 5-HT, and 5-hydroxyindoleacetic acid (5-HIAA), the breakdown product of 5-HT, were measured using high performance liquid chromatography.

RESULTS: After one week, a significant increase of Trp levels was observed in blood samples from mice treated with *B. subtilis R0179* when compared to PBS-treated mice. Interestingly, a marked decrease in 5-HT and 5-HIAA were measured in brainstem tissue of mice receiving the Trp-synthesizing bacteria compared to control mice. However, in the hippocampus, no changes in 5-HT but lower levels of 5-HIAA were found compared to control.

CONCLUSIONS: Trp-synthesizing bacteria in the gut increase blood levels of Trp and differentially modulate 5-HT signaling in the brain in unexpected ways (reducing 5-HT levels). It is possible that the effect on the serotonin signaling pathway observed after one week of treatment with *B. subtilis R0179* represents acute changes and longer time points will need to be studied. The mechanism underlying this modulation of central 5-HT signaling and the effects it may have on behavior remains to be determined.

Insulin signaling induces neuronal circuit restructuring during learning in C. elegans

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Connectomic studies of model organisms have been invaluable in dissecting the structure-function relationship of nervous systems. However, the limited sample size of these studies has impeded analyses into how experiences can bring about changes in circuit architecture and hence induces learning behaviors. In my postdoctoral work show, I showed that asymmetric salt-sensing circuit in the nematode *Caenorhabditis elegans* undergoes restructuring during salt associative learning (Tang 2023). Using iBLINC, fluorescent- based technique of labeling specific synapses, we were able to visualize and quantify the number of synapses between the salt sensing neurons ASE and odor sensing neuron AWC. We found that in naïve worms, which prefer the low salt concentration they experience in the presence of food, had a left-biased neural network architecture, i.e. the connections between ASE and AWC on the left are stronger than that on the right. However, animals conditioned at elevated salt concentrations change this left-biased network to a right-biased network, which occurs through addition of new synapses. Through mutant analysis, fluorescent reporter, and cell-specific knockout, we demonstrated that this change in circuit structure is in response to asymmetrical insulin signaling. Therefore, experience-dependent changes in an animal's connectome are induced by insulin signaling and are fundamental to learning and behavior. We intend to further explore the relationship between insulin signaling, synapse regulation, and learning. Firstly, we are investigating the role of insulin-like peptide binding proteins (IGFBPs) in neuronal functions through salt preference assay of mutant animals. Secondly, we are trying to identify genes that are under insulin-induced transcriptional control and regulate synapse formation and elimination through cell-specific RNA profiling. With these approaches, we hope to gain insights into the general principles in the neurobiology of learning.

Afferent-specific neuromodulation of excitatory synaptic transmission in the prefrontal cortex

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Neurotransmission is regulated by a myriad of pre- and postsynaptic signaling mechanisms, including ubiquitous presynaptic G-protein-coupled receptors that modulate transmitter release at individual boutons. In the mouse neocortex, receptors for acetylcholine (ACh) and serotonin (5-hydroxytryptamine, or 5-HT) are expressed pre- and postsynaptically in neuron-subtype-specific patterns. Postsynaptically, 5-HT inhibits extratelencephalic (ET, or corticofugal) neurons that project to the brainstem but increases the gain of intratelencephalic (IT, or corticocortical) projection neurons. Conversely, postsynaptic muscarinic ACh receptors (mAChRs) preferentially increase the gain of ET neurons. We used optogenetic isolation of excitatory corticocortical and thalamocortical afferents to the mouse prefrontal cortex to map the connectivity of corticocortical and thalamocortical afferents in layer 5 IT and ET neurons. We find that corticocortical projections from the contralateral hemisphere robustly target both IT and ET neurons, and glutamate release from these terminals is sensitive to both acetylcholine (acting at M4-type mAChRs) and 5-HT (acting on 5-HT$_{1b}$ receptors). On the other hand, afferents from the mediodorsal nucleus selectively target IT neurons and are insensitive to presynaptic modulation by either ACh or 5-HT. These results reveal afferent-specific targeting and presynaptic regulation of glutamatergic transmission in the mouse prefrontal cortex.

Intestinal colonization of *Akkermansia muciniphila* exacerbates EAE dependent on the microbiota context

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Multiple Sclerosis (MS) is autoimmune disease of the central nervous system (CNS) in which myelin-reactive immune infiltration contributes to neuronal, neurodegeneration, and subsequent disability. Various studies have documented elevated abundance of the gut bacteria *Akkermansia muciniphila* among patients with MS and animal models of MS compared to healthy control subjects, insinuating it may be a risk factor for disease. However, *A. muciniphila* is widely considered beneficial in various other pathological contexts, and recent studies suggest that *A. muciniphila* may be associated with reduced disability score in MS. Short-chain fatty acids (SCFAs), a category of bacterial metabolites with immune consequences for the host, are modulated by *A. muciniphila* and represent a potential pathway in which *A. muciniphila* may modulate MS. To better understand the role of *A. muciniphila* in MS, we have generated two distinct microbiome models within C57BL/6J mice with and without *A. muciniphila*, providing a variety of ecological contexts in which *A. muciniphila* may behave differently. Using experimental autoimmune encephalomyelitis (EAE) to model MS, we assessed the impact of *A. muciniphila* colonization on disease severity across microbiome models and identified a microbiome in which the addition *A. muciniphila* leads to increased disease severity. Exacerbated EAE severity in *A. muciniphila*-colonized mice was associated with increased Th17 responses and an increased frequency of CNS-infiltrating immune cells. We performed 16S sequencing of fecal samples to identify differences in the gut microbiome by *A. muciniphila* colonization. We observed a reduction of gut Clostridia, key producers of the SCFA butyrate, concomitant with *A. muciniphila* colonization in our microbiome model in which *A. muciniphila* exacerbates EAE. Likewise, analysis of inferred functional pathways relevant to SCFA metabolism highlighted 6 pathways related to reduced butyrate production. Taken together, our data contribute to the complex role of *A. muciniphila* on the severity of CNS autoimmunity.

Offspring From Preeclamptic Pregnancy Have Worse Stroke Outcome in Adult Males but Not Females

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Introduction: Offspring from preeclamptic women are at increased risk of neurologic disorders later in life and have an increased risk of death from stroke. We hypothesized that the unfavorable intrauterine environment during preeclampsia (PE) causes cerebrovascular dysfunction and inflammation in offspring that worsens stroke outcome.

Methods: Adult male and female offspring (n=6-8/group) from Sprague Dawley rats that had normal pregnancies (NormP-F1) or experimental PE (ePE-F1) underwent transient MCA occlusion (tMCAO) for 3 hours with 1 hour of reperfusion. Blood gases were kept within normal ranges. Multisite laser Doppler was used to measure changes in pial collateral flow and MCA cerebral blood flow (CBF) simultaneously. Infarct and edema were measured by TTC staining. After tMCAO, plasma was obtained to measure circulating inflammatory factors using Multiplex and ELISA. Data are reported as mean±SEM.

Results: Offspring from ePE dams had elevated blood glucose, and sex-specific increases in infarct and edema—only ePE-F1 males had significantly larger infarcts compared to all other groups (48.0±5.7% vs. 9.0±1.3% ePE-F1 female, 10.9±3.9% NormP-F1 male, and 4.9±1.9% NormP-F1
female). The rate of hemorrhagic transformation (HT) was increased in both ePE-F1 males (100%) and females (62.5%) vs. NormP-F1s (male: 37.5%, female: 0%). The increased infarct in ePE-F1 males was associated with poor collateral flow, decreasing -59.8±3.3% which remained low during the ischemic period. In contrast collateral flow decreased -43.9±17.8% in ePE-F1 females that was not sustained and increased to baseline after 40 min of tMCAO. Both female and male NormP-F1's had robust collateral flow, decreasing only -14.8±25.5% and -17.5±22.1%, respectively. There was an increase in circulating VEGF in ePE-F1 males vs NormP-F1 males (188.8±69.3 vs 39.2±7.5 pg/ml; $p<0.05$) but not between females. The concentration of the proinflammatory cytokine IL-1β and the anti-inflammatory cytokine IL-10 were increased in ePE-F1 males vs. NormP-F1 males (119.3±17.8 vs 35.3±7.7 and 765.4±83.8 vs 356.4±62.6 pg/ml; $p<0.05$) indicating an inflammatory mechanism not present in other groups.

Conclusion: These data demonstrate the far-reaching effects of adverse pregnancy in PE that worsens stroke outcome in offspring. The mechanisms by which PE negatively impacts offspring in a sexually dimorphic manner is unclear but may be related to (epi)-genetic programming in utero on cerebrovascular development.

Subthreshold electric fields bidirectionally modulate neurotransmission through axon polarization

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Subthreshold electric fields (E-fields) fail to directly stimulate neuronal firing but have been shown to powerfully modulate ongoing brain activity. While E-fields are known to polarize membranes, the mechanisms by which individual neurons respond to subthreshold E-fields remain unclear, especially at the subcellular level within axons. Previous experimental work showed somatic and dendritic polarization by subthreshold E-fields alters postsynaptic integration, but the effects of subthreshold E-fields on axonal polarization and presynaptic transmission have not been measured. This is primarily due to the inaccessibility of fine axon terminals to electrical recording techniques. Here, we used genetically encoded optical indicators of voltage, glutamate, and calcium to measure the subcellular effects of low intensity, spatially uniform electric fields in cultured hippocampal neurons. Voltage imaging revealed E-fields generate a biphasic polarization distribution across neuronal morphologies, with depolarization at the cathodal and hyperpolarization at the anodal end, in agreement with compartmental modeling predictions. By imaging glutamate release (iGluSnFR3) in individual presynaptic boutons, we found subthreshold E-fields exerted facilitation or suppression of glutamate release, depending on polarity. Facilitation had a nonlinear dependence on intensity, with over 70% of synapses significantly facilitated at 0.5 and 1 mA, while 5% were facilitated at the lowest intensity tested (0.1 mA). Most models of subthreshold modulation of synaptic transmission predict axonal depolarization broadens the AP waveform and enhances calcium influx, which subsequently increases the vesicular release probability. However, we did not observe significant changes in AP width using voltage imaging (Archon1) or peak calcium influx using calcium imaging (jGCaMP8f) in the axon during E-field stimulation. E-field induced facilitation had no effect on paired pulse ratio and was diminished by EGTA, suggesting subthreshold depolarization rapidly increased the size of the readily releasable pool via small changes in basal calcium levels. Our results suggest that subthreshold E-fields cause slight changes in axonal calcium channel gating kinetics and calcium influx, exerting bidirectional control on neurotransmitter release. In addition, this work establishes a platform for studying acute effects of electric fields on single neurons at the subcellular level.

Chronic Neuroendocrine Stress on Fear and Anxiety

Sarah Van Horn, Benedek Erdos

Our lab has developed a rat model of chronic neuroendocrine stress by overexpressing brain-derived neurotrophic factor (BDNF) with an AAV vector in the paraventricular nucleus (PVN) of the hypothalamus but had a limited understanding of the behavioral effects of the model. BDNF expression in the PVN increases during acute and chronic stress and regulates the activity of the hypothalamic-pituitary-adrenal (HPA) axis and sympathetic nervous system (SNS). By overexpressing BDNF in the PVN, we induce chronic activation of these pathways without introducing behavioral stressors, thereby separating neuroendocrine and behavioral stress to investigate the long-term effects. Three behavioral experiments investigated the expression of fear and anxiety behaviors in the PVN-BDNF model: a) light-enhanced acoustic startle, b) two days of the elevated plus maze, and c) fear conditioning with renewal. We found that the acoustic startle response is higher at baseline and more potentiated by light in the PVN-BDNF rats than in the GFP controls. Locomotor activity and exploration were higher in the PVN-BDNF rats, and they spent more time in the closed arm on the second day in the elevated plus maze. Locomotor activity and alternative defensive behaviors were higher for the PVN-BDNF rats in the fear conditioning experiment and both groups showed renewal of the conditioned freezing response. The results suggest that the PVN-BDNF model exhibits active defensive strategies in the presence of anxiety or fear-provoking stimuli. Overexpressing BDNF in the PVN appears to modulate the bed nucleus of the stria terminalis (BNST) and amygdala-dependent responses to threats, as light-enhanced startle is more BNST-dependent and fear conditioning is more amygdala-dependent. The behavioral differences implicate several regions that will later be explored with immunofluorescence, and the results contribute to our understanding of the role of stress-induced activation of the PVN in behavior.

DATA BLITZ

Mitochondrial Transfer in Diffuse Midline Glioma

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Diffuse midline glioma (DMG) is a deadly brain tumor that occurs primarily in children. Due to its location, surgical resection is often impossible, leading to a reliance on chemotherapy and radiation. However, neither is effective, with even promising chemotherapies such as the small molecule imipridone ONC201 showing high resistance rates over time. With a current two-year survival rate of <10%, it is clear that this cancer needs better treatment options as well as a deeper understanding of the mechanisms of drug resistance. Recent studies have identified the transfer of intact mitochondria as a contributing factor to chemoresistance in various cancer cell types, including glioblastoma. While this transfer has been demonstrated in other gliomas, it has not yet been investigated in DMG. Using flow cytometry and cell lines with fluorescently tagged mitochondria, we show that mitochondria can be transferred between DMG cells and neurons, providing the first evidence of mitochondrial transfer in DMG. Given that ONC201 alters cellular metabolism and increases the production of mitochondrial ROS in DMG cells, we hypothesize that mitochondrial transfer may contribute to ONC201 resistance, alongside other potential impacts on the cells' metabolism and proliferation.

Artificial sweeteners differentially activate sweet and bitter gustatory neurons in Drosophila melanogaster

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Artificial sweeteners are highly sweet, non-nutritive compounds that have become increasingly popular over recent decades as a strategy to reduce caloric intake while maintaining palatability. While these additives are considered biologically inert and safe for consumption, their long-term impacts on health remain unclear and controversial. Many of these concerns are related to the unique sensory attributes of artificial sweeteners, which can be anywhere from 200-700 times sweeter than natural sugars. Moreover, there is evidence suggesting that these chemicals also interact with bitter taste receptors, implying that artificial sweeteners may represent a more complex chemosensory signal. The fruit fly, *Drosophila melanogaster*, has been a critical model organism for studying the effects of artificial sweetener exposure on many phenotypes. However, less is known about the basic sensory characteristics of these chemicals and how these signals might affect feeding. Therefore, we describe the behavioral taste responses to a panel of popular artificial sweeteners using the proboscis extension response (PER) assay and found variable, concentration-dependent responses to the different tastants. Next, we performed *in vivo* calcium imaging to record sweetener-induced taste responses in both sweet and bitter gustatory receptor neurons (GRNs), two populations that reciprocally impact feeding behavior. We found that sucralose and rebaudioside A (Reb A) both co-activate sweet and bitter GRNs, while aspartame only activates bitter GRNs. To determine the behavioral impacts of sweet and bitter co-activation, we again used PER to show that low concentrations of sucralose signal appetitive feeding while high concentrations signal feeding aversion. Finally, optogenetic and constitutive silencing of bitter GRNs reduced the aversive signal elicited by high concentration sucralose and significantly increased sucralose feeding behavior. Together, we conclude that artificial sweeteners generate a gustatory signal that is more complex than “sweetness” alone, and this bitter co-activation has behaviorally relevant effects on feeding that may help flies flexibly respond to these unique compounds.

ABC renewal of conditioned fear following extinction and counterconditioning in male and female rats

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Pavlovian extinction is a method of conditioned response reduction and occurs when the conditioned stimulus (CS) is repeatedly presented in the absence of the unconditioned stimulus (US). However, when the CS is experienced in a context that is different from the extinction context, there is a recovery of the conditioned response, a phenomenon known as renewal. There has been suggestion that renewal of extinguished responses is a sex-specific process, however, this has been questioned by recent findings (Schoenberg et al., 2024) where females renew in both ABA and AAB renewal designs. Here we assess renewal in a third context (ABC renewal) following either extinction or a second method of response reduction: counterconditioning, in which fear responding to the initial conditioning is decreased via counterconditioning the original CS to delivery of an appetitive stimulus. In this experiment, we directly compared the renewal effect following extinction vs counterconditioning and found no evidence of a sex difference in either. Additionally, although counterconditioning produced a faster reduction of fear responding, there was no evidence that the renewal effect differed based on treatment.

POSTER

Poster 1

Recurrent Early Life Seizures On Corticohippocampal Signaling Development: Post-seizure maturation as a novel circuit dysfunction mechanism

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Hippocampal theta and gamma oscillations play a critical role in learning and memory. However, it is unclear how the generation of these oscillations correlates with corticohippocampal (eg, CA1, MEC3-SLM, DG LEC2-OML/MEC2 MML) synaptic-dendritic development. This precludes a mechanistic understanding of how early-life seizure (ELS) alters circuit maturation, hippocampal-entorhinal coordination, and post-seizure cognitive outcome. We hypothesized ELS disrupts network-level maturation, significantly altering oscillatory spectral properties (power and frequency), current source density (CSD), and phase-amplitude coupling (PAC) at specific dendritic compartments.

We used high-density laminar silicon probes (H3, Cambridge Neurotech) to compare hippocampal CA1 and dentate gyrus (DG) theta and gamma spectral properties and PAC in juvenile (P21) and adult (P90) rats, with or without ELS. ELS induction was achieved through the recurrent flurothyl seizure induction model (seizures induced at P9-13, 5 seizures/day). This approach enabled us to measure synaptic-dendritic changes as a function of control maturation and post-seizure maturation.

We demonstrate spectral power, CSD, and PAC changes in the period between ELS induction and P21, and seizure impacts into adulthood. Control adults differ in spectral properties relative to control juveniles, with much greater theta and gamma amplitudes. Relative to control juveniles, CSD analyses also showed significant post-seizure changes with lower OML activity in ELS juveniles. In ELS adults, maturation precipitates broad DG activity depression with significant shifts in corticohippocampal coordination at OML and MML synaptic inputs relative to slow theta (3hz) and slow gamma (40hz) PAC. In contrast, control maturation underlies increased CSD activity in the stratum radiatum and SLM, theta and gamma spectral property changes, and slow-theta gamma coupling at SLM, OML, and MML relative to all other groups. We propose ELS impairs corticohippocampal circuit throughput.

Our results are consistent with prior work, suggesting theta and gamma signal properties are network efficacy proxies at specific dendritic compartments that may be necessary for decoding DG corticohippocampal inputs. Our results also suggest post-seizure dysmaturation, disentangling short-term and long term seizure impacts as development functions. This sheds new light on seizure consequences in the developing brain and may open previously unforeseen treatment windows.

Poster 2

Integrating Multimodal Data to Understand Drug-Resistant Epilepsy

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Patients with drug-resistant epilepsy often undergo surgical intervention, which offers access to a wealth of diverse data, including intracranial EEG (iEEG) from monitoring, neuropsychological assessments, and histological samples. Despite the richness of these datasets, much of their potential remains untapped when analyzed in isolation.

This project will develop methods to bridge the gap between iEEG and spatial transcriptomics. A key goal is to identify electrophysiological features that correspond to cellular and molecular changes in brain tissue. Using feature selection techniques and classification approaches, we aim to distinguish between tissue types based on iEEG patterns—such as comparing regions within and outside of seizure onset zones and between epilepsy and other brain pathologies. If successful, linking iEEG and histology findings could allow for new diagnostic and prognostic biomarkers that avoid invasive sampling.

We also aim to explore links between cognitive deficits, as measured by neuropsychological testing, and underlying molecular pathways. By integrating these datasets, we hope to uncover patterns that connect tissue-level changes to functional impairments.

This work represents a step toward more comprehensive data integration in epilepsy research, with the potential to shed light on disease mechanisms and improve predictions of clinical outcomes.

Poster 3

Characterizing Network Inhibition and Cognitive Deficit in Kcnt1 Mice

Dylan Marchand, Eli Rachimi, John Hayward, & Jeremy Barry

Mice with mutation of the Kcnt1 gene has been implicated in a range of developmental seizure disorders with high rates of intellectual disability. We hypothesize that corresponding perturbation to Slack channels (Na^+ activated K^+ channels) preferentially affect somatostatin positive (SST+) interneurons, likely by making these critical cells hypoexcitable. This leads to the disinhibition of local circuits underlying the coordination of intercortical and corticohippocampal networks. Disinhibited networks, as a result of hypoexcitable interneurons, result in generalized seizures and learning and memory deficits.

To address this hypothesis, we used several approaches in headfixed wild-type and Kcnt1-YH (heterozygous and homozygous) mice to interrogate corticohippocampal networks on the CA1 and dentate gyrus somatodendritic axes, as well as putative frontal lobe – hippocampal interactions: 1) Behavior through active avoidance on a rotating arena; 2) Electrophysiological measures of local field potentials and SST+ interneurons and excitatory cells through high-density laminar silicon probes; 3) SST+ specific optogenetics; and 4) Simultaneous fast ultrasound of M2 and hippocampus.

Our preliminary data suggest that Kcnt1-YH mice demonstrate cognitive deficit phenotypes on the active avoidance task in which severity correlates with genotype (i.e., heterozygous vs homozygous). Likewise, we have found a relationship between genotype and the degree of the perturbation corticohippocampal circuits, where inputs underlying the generation of dentate spikes are transformed into hypersynchronous interictal epileptiform discharges. Preliminary results from optogenetic stimulation of SST+ interneurons responsible for regulating these inputs has shown that we can limit this circuit perturbation and significantly decrease interictal discharge discharge rate. Lastly, we will show that using the same headfixed approach, we can compare regional excitability and measure potential angioarchitecture difference in wild type or Kcnt1 mice via fast ultrasound.

Poster 4

Reversing Cognitive Deficits in the PTEN Knockout Model for ASD: From Behavior to in-vivo Electrophysiological Recording

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Abstract

Autism spectrum disorder (ASD) is a neuropsychiatric disorder without a clear understanding of how associated genetic mutations influence interactions between cell morphology, circuit function, and ultimately cognition and behavior. Loss of the PTEN gene is associated with a high risk for ASD as well as macrocephaly and epilepsy. In the animal model, PTEN KO in the hippocampal dentate gyrus (DG) results in dendritic overgrowth and somatic hypertrophy. Patch physiology results have shown these morphological alterations leading to hyperexcitability. We hypothesize that these changes to DG granule cells will disrupt corticohippocampal circuit function and lead to deficits in hippocampal dependent spatial behavior. In this study, we aimed to characterize network level effects of PTEN KO in relation to the percentage of granule cells KO'd in the DG. Moreover, we wished to characterize the effect this relationship had on the perturbation of the corticohippocampal circuit and corresponding cognitive deficit. To study the specific effects of PTEN KO on hippocampal circuit function, DG granule cells were coinfected with a cre-recombinase and DREADD viruses, allowing for selective inactivation of KO cells that have been in the network since early development (post-natal day 7). We tested the cognition and spatial memory of PTEN KO mice using the spatial accuracy behavioral task and hippocampal circuit function through high density electrophysiological probe recordings during head-fixation.

PTEN KO mice exhibited deficits in learning and spatial memory by requiring more training sessions than controls to reach criterion. With regard to network efficacy, 5/13 PTEN KO mice exhibited Inter-ictal discharges (IEDs), an indication of severe network hypersynchrony and putative epileptogenesis, while 8/13 did not. We refer to these two groups as PTEN High and PTEN Low respectively. Relative to controls, we found increased power and mean frequency in the bottom blade of the PTEN KO dentate gyrus in theta (3-12 Hz) and medium gamma bandwidths. In PTEN Low mice, we found a trend for decreased lower maximum power for theta, slow gamma, and medium gamma as well as higher mean medium gamma frequency. In accordance with these spectral changes, we've also found corresponding increases in Current Source Density (CSD) within the bottom blade of the DG predictive of IEDs In line with this finding, while injection of CNO in control animals does not affect behavioral performance , in PTEN KO animals CNO decreased 2nd granule cell layer to the level of controls and significantly decreased the IED rate, and significantly improved performance on goal rotation probe trials. These preliminary data show a disruption of hippocampal circuit function in PTEN KO mice and hippocampal dependent spatial behavior that we predict will correlate with the percentage of affected DG cells. We propose a mechanism which causes both chronic circuit perturbation of theta and the intermittent production of IEDs; the hypersynchrony of the granule cell layer. This discovery has implications for preventing epileptogenesis and cognitive deficits for ASD.

Poster 5

Autistic traits and visual language proficiency interact in comprehending visual narratives

Del Rosner, Kristina Fantoni, Neil Cohn, Emily Zane, & Emily Coderre

Pictures are often assumed to be “easier” for autistic individuals to understand compared to verbal stimuli. However, like verbal language, understanding picture stories (hereafter “visual narratives”) requires proficiency and relies on numerous cognitive skills (e.g., theory of mind, inference generation) that vary between individuals. While self-report measures of visual narrative experience exist, no standardized assessments of comprehrension have been developed. We aimed to develop the first objective assessment of visual narrative comprehension and explore whether scores are modulated by visual language fluency and/or autistic traits.

Autistic and non-autistic adults (*n*=46) completed a new measure of visual narrative comprehension consisting of comics in three difficulty levels (5 comics per level): 1) Easy: Garfield comics; 2) Medium: Peanuts comics; 3) Difficult: Calvin & Hobbes comics. For each comic, participants first arranged scrambled panels into a coherent story. We coded whether they arranged panels in the same order as the original comic and how often they identified the same two sequential panels as in the original. Participants then narrated their story and we coded how well they represented the main idea of the original comic. Finally, participants completed 8 comprehension questions by selecting one of two pictures that best answered a written question (correct/incorrect). Participants also completed the Autism Quotient (AQ), a self-report measure of autistic traits, and the Visual Language Fluency Index (VLFI), a self-report questionnaire assessing experience with visual narratives.

Overall comic comprehension was marginally associated with comic experience, providing preliminary evidence for our measure’s validity. Comic experience also interacted with autistic traits in the most difficult comics: VLFI was positively associated with comprehension for participants with lower but not higher AQ. These results suggest that proficiency is highly relevant for visual narrative comprehension and operates differentially for different levels of autistic traits. Thus, any use of visual narratives with autistic individuals needs to also consider visual narrative proficiency.

Poster 6

Inferencing During Visual and Verbal Narrative Comprehension in Autism: An EEG Study

Devon Kearns, Olivia Ciocca, Holly Chappell, Caitlyn Soong, Nicole Sperrazza, Emily Zane, Neil Cohn, & Emily Coderre

Autistic individuals sometimes struggle with understanding stories told verbally (i.e., through written/spoken language) and visually (e.g., comics). Inferencing abilities help comprehenders interpret implicit information by filling in the gaps between explicit events in both visual and verbal modalities. Autistic individuals sometimes show difficulties with inferencing, which could contribute to comprehension difficulties in autism that should extend to both verbal and visual narrative comprehension. However, no studies have directly compared inferencing in autistic individuals across modalities.

We collected EEG data from 52 participants (mean age=25, range=18-65) with a range of autistic traits (measured by the Autism Quotient: M=21, range=4-44) during two inferencing tasks. In the visual domain, participants viewed 6-panel comic strips (normal condition) or 5-panel strips in which the panel depicting the narrative climax was removed (inference condition). In the verbal domain, participants read 5-sentence stories (normal) or 4-sentence stories in which the climactic sentence was dropped (inference). Participants also completed measures of visual language fluency and reading comprehension.

In the visual modality, fluency interacted with autistic traits in late time windows (900-1000 ms). With high visual language fluency, the level of autistic traits did not impact inferencing abilities; when fluency was low, greater autistic traits were associated with larger positivities. In the verbal modality, higher autistic traits were associated with larger early negativities (200-300 ms) regardless of reading comprehension scores, possibly indicating an earlier onset of inferencing processes.

Overall, these results suggest that autistic traits influence inferencing processes during narrative comprehension, albeit in different ways across modalities.

Poster 7

Characterizing the Structure and Relationship Between Activity Participation, Mental Health, and Neurocognition of Youth Ages 8-11 in the ABCD Study

Annabel Diestel, Nicholas Allgaier, Hugh Garavan, Alexandra Potter

Background/Aims:
Extracurricular involvement is often an important context in which adolescents develop. The present study first aims to utilize baseline data to characterize the structure of activity involvement in the Adolescent Brain Cognitive Development study. The second aim of this study is to investigate how the structure of activity involvement relates to mental health outcomes and neurocognition.

Methods:
The Adolescent Brain Cognitive Development Study Baseline (data release 5.1) data was used for analysis (n = 8,882; 47.83% female; ages 8-11). A principal component analysis (PCA) was performed using the number of years of parent-reported youth participation in 31 activities assessed at the baseline visit. Each participant was then scored on each of the components found. Linear mixed effects models were used to examine the relationship between the derived components and Total Problems from the Child Behavior Checklist (CBCL) Syndrome Scale, and the Cognition Total Composite score from the NIH Toolbox. The first ten principal components were used in the regression. Covariates included sex at birth, age (months), pubertal stage (youth-reported), and combined household income. Participants were nested within families and collection site as random effects.

Results:
We found that the number of years youth had participated in activities had 31 total principal components, 81% of the variance in years of activity participation accounted for by the first 10 components.

The results of the linear mixed model for total problems on the CBCL suggest that the overall model was significant ($p < .001$). For the NIH Toolbox Total Cognition Composite Scores, our model was also found to be significant ($p < .001$). Pc1, pc3, and age significantly positively predicted total mental health problems ($p < .05$). Pc2, pc4, pc5, pc9, pc10, sex, and income significantly negatively predicted mental health problems ($p < .05$). Cognition was significantly positively predicted by pc1, pc2, pc4, pc5, pc6, pc7, income, age, and pubertal stage ($p< .05$). Pc3, pc8, and pc10 significantly negatively predicted cognition ($p < .05$).

Conclusions and Limitations:
The results of the present study showed that years of activity participation for youth at the baseline visit of the ABCD Study can be characterized by 10 components, and that these components are significantly related to CBCL total problems and NIH Toolbox Total Cognition. We found that the components relate to mental health and cognitive outcomes with variability in both strength and direction of effects. Our study only included baseline data, so future investigation will be necessary to understand how these relationships may change over time. These findings help us understand how youth activity participation may relate to mental health and neurocognition and set the stage for longitudinal analysis of these relationships.

Poster 8

The relationship among smartphone addiction, social media addiction and social intelligence

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Excessive smartphone use (or smartphone addictions) may be related to impairments in social/emotional intelligence in college students. The present study assesses the effects of excessive smartphone use on social skills and relationship quality within this population. Students completed a smartphone addiction survey (SAS), and measures of social intelligence, FOMO, emotion regulation, social media addiction, and friendship quality. There was a positive correlation between SAS and social media addiction. For social functioning, both smartphone and social media addiction were positively correlated with FOMO. However, only social media addiction showed a direct negative correlation with social intelligence ($p < .06$). These findings replicate known relationships among smartphone use, FOMO, and social media addiction. Even though a strong correlation with social intelligence was not seen for either SAS or social media addiction, the social anxiety that is associated with FOMO may suggest some degree of impaired social function. Excessive smartphone use is associated with negative psychological outcomes, such as anxiety, depression, and addiction, which include impairments in social intelligence. Accordingly, our preliminary results suggest adverse relationships may exist among smartphone addiction, social media addiction, and social intelligence. Additional data will be needed to confirm and further explore these relationships.

Poster 9

Neural Markers of Methylphenidate-Induced Behavioral Inflexibility: LFP-Based Prediction and Path Analysis via Dynamic Time Warping

Matthew Company, Lucas Dwiel, Moriah McGuier, Elise Bragg, and Wilder Doucette

Neural oscillations hold promise as biomarkers of treatment outcomes in psychiatric disorders. To test this potential, we trained rats to perform a Delay Discounting Task (DDT) with simultaneous local field potential (LFP) recordings and administered well-researched interventions (pharmacological and brain stimulation) to instigate changes in DDT behavior. Initially, we sought to determine whether machine learning models (LASSO) trained on LFPs could accurately predict and detect intervention-induced behavioral changes. Methylphenidate (MPH) was chosen as a pharmacological intervention and found to display delay-presentation-dependent effects (i.e., increasing delays throughout a session biases animals toward delayed choices, whereas decreasing delays biases toward immediate choices), suggesting MPH does not impact delay sensitivity but rather another decision-making domain—hypothesized to be behavioral flexibility.

To assess this hypothesis, we trained separate machine learning models to predict unidirectional decreases in delay discounting (mean AUROC = 0.942) and to predict bidirectional changes in delay discounting (mean AUROC = 0.74). These models indicate that MPH-induced LFPs contain neural correlates of both delay-presentation-dependent signaling and behavioral inflexibility. To further probe whether MPH decreases behavioral flexibility, we applied dynamic time warping, a measure of path similarity, to DeepLabCut-generated position data extracted from videos recorded during operant behavior.

Overall, our findings indicate that MPH-induced changes in LFPs capture neural correlates of MPH-driven behavioral inflexibility, and we test new analytic methods for assessing behavioral flexibility. These approaches hold promise for translational research aimed at advancing personalized medicine in psychiatry.

Poster 10

Screen Time and Mental Health: Exploring Mobile Phone Use in Trauma-Exposed Individuals

Jennifer Ha, Johanna Hidalgo, Natalie Noble, Matthew Price

Designing effective digital interventions for individuals with trauma-related psychopathology requires understanding how they use their mobile devices. This study (N = 332) examined the relationship between the frequency of mobile phone activities and PTSD symptom severity in trauma-exposed individuals. It was hypothesized that greater time spent on gaming and social media would correlate with higher psychopathology. Participants completed measures of trauma exposure (LEC-5), PTSD symptoms (PCL-5), and mobile activity frequency.

A 2x4 (Gender x Frequency) ANOVA revealed significant main effects for time spent on mobile games ($F = 4.15$, $p = .013$), texting ($F = 6.28$, $p = .001$), listening to music ($F = 3.05$, $p = .043$), and watching videos ($F = 7.89$, $p = .0003$), with greater usage associated with higher PTSD symptoms. An interaction effect was found for social media use and gender ($F = 4.60$, $p = .022$): increased social media use was linked to elevated PTSD symptoms in females but not males.

These findings indicate that frequent engagement in specific mobile phone activities is associated with heightened PTSD symptomology, with gender differences evident in social media use. Understanding these patterns can inform the design of trauma-informed digital interventions by leveraging activity-specific engagement to support mental health.

Poster 11

Associations between trauma exposure and perceptions of conflict within the family system

Emma Renwick & Alexis Brieant; University of Vermont

Many family systems are affected by trauma exposure, which can contribute to increased family conflict between youth and their caregivers (Stewart et al., 2023). Perceptions of conflict may vary between family members due to differences in age and cognitive development, both of which may impact interpersonal skills, including conflict resolution (Van Doorn et al., 2011). We hypothesized that higher levels of trauma exposure would be associated with higher levels of perceived family conflict for both youth and their caregivers, and that the effects of trauma would be stronger for youths' perceptions of conflict relative to caregivers' perceptions. Finally, we explored whether family income moderated these associations given evidence that low socioeconomic position can exacerbate the effects of trauma (Santiago et al., 2009). Data were drawn from the Adolescent Brain Cognitive Development (ABCD) Study (n = 11,868), a longitudinal study of youth development in the United States (9-10 years old at baseline). At baseline, youth lifetime trauma exposure was measured using the K-SADS interview and caregivers reported on their total annual household income. At the year 3 follow-up (ages 12-13), perceptions of family conflict were measured using the Family Conflict Subscale of the Family Environment Scale, which was completed by both the caregiver and the child. A multivariate regression model was used to estimate the effect of trauma exposure on youth and caregiver perceptions of family conflict, as well as the interaction effect of trauma and family income level (see Figure 1). Consistent with our hypotheses, the results indicated that higher levels of trauma exposure were associated with higher levels of perceived family conflict for both youth (ß = .06, *SE* = .06, *p* = .01) and their caregiver (ß = .08, *SE* = .06, *p* = <.001). The effect sizes for the outcomes on youth versus caregiver perception of family conflict were comparable. The interaction between trauma and income was non-significant (*p* > .05) for both youth and caregiver perceptions, suggesting that, regardless of income level, trauma had a significant effect on perceptions of family conflict. Altogether, these findings suggest that families experiencing traumatic events, regardless of income, could benefit from supports and interventions to minimize and prevent conflict within the family system.

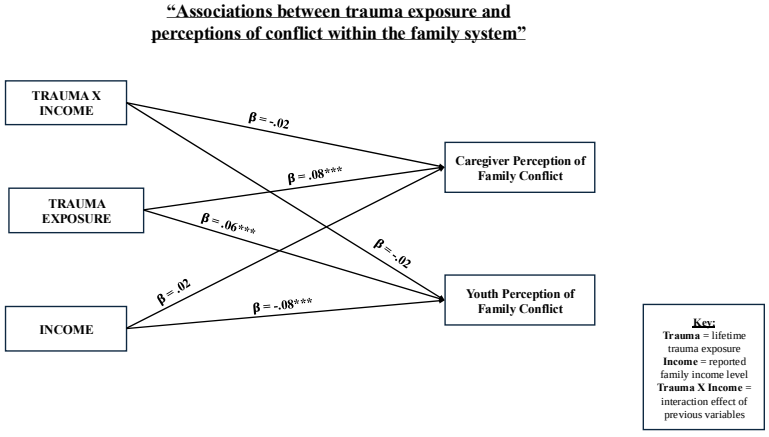

Figure 1. Standardized estimates for the effects of trauma exposure, income level, and their interaction on youth and caregiver perceptions of family conflict. *Note:* **p* < .05, ****p* < .001

Poster 12

Influence of context on extinguished appetitive conditioning in male and female rats

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Extinction is fundamental to adaptive behavior, in that it allows organisms to alter previously conditioned behaviors based on the prevailing environmental contingencies. Extinguished responses, however, will renew when the conditioned stimulus (CS) is presented outside the extinction context. There has been some suggestion that renewal after extinction of appetitive conditioning is a sex-specific process, with only male rats showing renewal (e.g., Anderson & Petrovich, 2015, 2017, 2018). The purpose of the present experiments was to revisit the role of sex in appetitive renewal, in part because an earlier literature demonstrated renewal in experiments with only female rats (e.g., Bouton & Brooks, 1994). In three experiments, rats underwent appetitive Pavlovian conditioning in Context A, followed by extinction in Context B, and then within-subject renewal testing in both B and A. In Experiment 1a, renewal was present for both male and female rats. In Experiment 1b, the procedure included exposures to Context A during the extinction phase. Once again, renewal was observed in female rats. In Experiment 2, we assessed if cycling hormones contribute to renewal in female rats. To do so we compared intact female rats with ovariectomized (OVX) female rats, and observed robust renewal in both groups. Our results support the notion that renewal is a general behavioral phenomenon, and is one reason why behavior change may be difficult to sustain (Bouton, 2014).

Poster 13

Conditioned reinforcement based on real appetitive outcomes in humans

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Substance use disorders are chronic relapsing conditions driven by learning processes. Drug-paired stimuli initiate cravings and promote drug-seeking behaviors. One of the potential ways that drug-paired stimuli influence behavior is through conditioned reinforcement. There is limited understanding of how conditioned reinforcers can be addressed in human laboratory settings. We developed a new method for establishing a conditioned reinforcer using real appetitive outcomes. Participants were recruited from the UVM undergraduate psychology research pool. The experiment consisted of brief presentations of four symbols (A, B, X, and Y) on a computer monitor. After presentations of a symbol compound (AX and AY, BX and BY) participants rated their expectation of receiving an M&M and were then told whether or not they received an M&M. Training consisted of 36 AX+/AY+ trials followed by an M&M intermixed with 36 BX-/BY- trials followed by nothing. A test phase followed in which a joystick was introduced and could be moved left or right to produce brief presentations of A or B. M&Ms were not available in the test. Results suggested that participants learned which symbols predicted M&Ms and which did not. Liking ratings were also higher for A than B. In the test, participants moved the joystick to produce A significantly more than B, suggesting that A acquired a rewarding value for the specific joystick movement consistent with conditioned reinforcement. The method developed in this study can be used to examine additional methods aimed at changing the conditioned reinforcing properties of reward-paired stimuli.

Poster 14

Robust renewal after extinction of remotely acquired Pavlovian conditioning

Charles, J. W., Moriarty, S. K., Winterbauer, N. E., Cooper, E. A., & Todd, T. P.

Aversive experiences can be encoded as long-lasting memories through Pavlovian fear conditioning, that when later retrieved can elicit a host of emotional responses. These responses can be reduced via extinction, in which the Pavlovian conditioned stimulus (CS) is repeatedly presented in the absence of the aversive unconditioned stimulus (US). The resulting extinction memory is highly context dependent, and further exposure to the conditioning stimuli outside of the extinction context can cause renewal of fear memory related behavior. While we know that the brain regions responsible for the storage and retrieval of fear memory change over time, little is known about extinction when the interval between conditioning and extinction is fairly long (e.g., weeks to months). In this study, we examine ABA (Experiment 1) and ABC (Experiment 2) renewal, with either a 1-day (recent) or 27-day (remote) retention interval to test how extended intervals influence the extinction and renewal of Pavlovian fear conditioning. Robust renewal was observed in both Experiments 1 and Experiment 2. Further, the size of renewal was not modified by the age of the original memory. Thus, even though remote memories require unique brain regions for their retrieval, learning and behavior processes that govern extinction and renewal are not altered.

Poster 15

A novel context disrupts the expression of conditioned fear

Rocco, E. A., Moriarty, S. K., Waycott, O. S., Winterbauer, N. E., & Todd, T. P.
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Intro: Pavlovian fear extinction is remarkably context specific. That is, although extinction results in a reduction of previously acquired conditioned responding, such a reduction is not permanent. Indeed, previously conditioned behaviors are apt to return under a variety of circumstances, such as a change in context. The return of extinguished behavior outside the extinction context is referred to as *renewal*. A recent prior study (Binette et al., 2022), reported renewal in male, but not female, rats. However, in this experiment, testing occurred in a completely novel context. We reasoned that the novel context may have disrupted renewal, and therefore sought to test this within a single experiment. To test this hypothesis, we conducted an experiment in which we manipulated the exposure of female rats to a novel context during the extinction phase and then assessed the renewal of conditioned fear in both familiar and novel contexts.

Methods: Two groups of rats (n = 16 per group) first received tone-shock pairings in Context A, resulting in a tone-shock association. Next, extinction occurred in Context B. During this phase, the tone was repeatedly presented alone in Context B, and freezing declined. In addition, during this phase, one group of rats (Exposure) was equally exposed to Context C. A second group of rats was never exposed to Context C but received equivalent handling (Novel). Specifically, Group "Novel" were placed in the carrier, brought into the lab, and then wheeled back to their cages.
After the extinction phase, within-subject renewal testing occurred in Context B and C, in which the tone was presented 5 times with no shock. The aim of renewal testing was to assess the rats' freezing behavior, which would provide insight into how the context influenced the retrieval of the conditioned fear response. The order of testing, meaning either Context B followed by Context C, or vice versa, was counterbalanced across rats. Following renewal testing, we also assessed freezing to a second target cue, that was not extinguished. This cue was first conditioned in A, then tested in B and C.

Results: During renewal testing, there was a robust renewal of freezing in group Exposure, however, renewal was eliminated for the Novel group. Interestingly, the Novel group also showed a reduced freezing response in Context C when tested with the second, unextinguished cue, suggesting that exposure to a novel context may not only disrupt the renewal of conditioned fear responses but also interfere with general expressions of fear behavior.

Discussion: Our results indicate that a novel context disrupts the expression of conditioned fear in female rats. Perhaps the most parsimonious explanation of the data is that novel contexts elicit unconditioned behaviors, such as exploration or other unconditioned fear responses, that compete with the freezing response. The lack of renewal in the Novel group and the reduced freezing response to the unextinguished cue further suggest that novel contexts may elicit a more generalized interference effect, diminishing the expression of conditioned fear across both extinguished and non-extinguished stimuli. Future directions will be discussed.

Poster 16

Freezing to distant and proximate contextual threat in male and female rats

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The ability to accurately associate environmental information with danger through contextual fear conditioning is critical for survival. Bolles (1970) highlighted the tendency for animals to develop species-specific defense reactions (SSDRs) to enable rapid and reliable engagement of the optimal physiological and behavioral responses to such threats. However, the threat being represented may exist anywhere along multiple spectra of physical, temporal, and psychological imminence. Corresponding SSDRs may thus represent anxiety-like behavior in response to distal or ambiguous danger, or fear-like/panic-like behavior in response to immediate threat, even while presenting as the same behavior (i.e., freezing). Special interest has been focused on the bed nucleus of the stria terminalis (BNST) as a primary mediator of anxiety-like responding. Hammack et al. (2015) found that pretraining lesions of the BNST in female Wistar rats attenuated freezing in a context that predicted a footshock unconditioned stimulus (US) at 10 minutes after exposure, while having no significant impact on freezing to a context that predicted shock at 1 minute. A similar effect specific to long context-US interval was observed by Goode et al. (2020) after temporary pharmaceutical inactivation of the BNST prior to test in male Long Evans rats. We investigated conditioning and extinction of freezing behavior across each timespan via the Goode et al. (2020) paradigm in both female and male Long Evans rats. We found that males learned extinction more gradually than females in both the original and a novel context [$p<0.05$ (sex); $p<0.01$ (sex*session)], regardless of context-US interval and despite no apparent sex difference in initial fear/anxiety learning. Future experiments will serve to clarify the role of the BNST in such responses via chemogenetic and pharmaceutical intervention, and to probe potential sex differences in effects of prior stress on anxiety-like behavior.

Poster 17

Do Thalamic Nuclei Contribute to the Effects of Context on Operant Learning?

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Contextual cues, background physical (e.g., olfactory, visual, auditory, and tactile sensations) and internal (e.g., hunger, stress) stimuli, modulate learned behaviors (Bouton, 1993, 2019). For example, rats may learn that an operant response leads to an outcome in one context (Context A), then, in a second context (Context B), the response no longer produces the outcome and the response extinguishes. When the rat is returned to Context A, the extinguished response returns (ABA renewal) (Bouton & Bolles, 1979; Bouton et al., 2011). We have shown that pharmacological inactivation of a region of the medial prefrontal cortex, the prelimbic cortex (PL), selectively reduces operant responding in Context A. Thalamic nuclei are directly connected with the PL and modulate its function. Here, we investigated two thalamic nuclei, the nucleus reuniens (RE) and the mediodorsal nucleus (MD), that have strong reciprocal connections with the PL (Vertes et al., 2015). We predicted that these regions would also be important for Context A operant responding. For the first experiment, rats underwent surgery to implant a single guide cannula into the nucleus reuniens. Animals underwent daily training sessions in which they were reinforced for lever pressing in Context A and were exposed to a second, untrained context (Context B). Rats were then tested in Contexts A and B after an intra-RE infusion of either muscimol, a $GABA_A$ receptor agonist (which temporarily inactivates neurons by hyperpolarizing them), or vehicle. RE inactivation reduced responding in Context A but not in Context B. On the following days, rats underwent extinction in Context B and were exposed to Context A (no lever present). After extinction training, rats received a second test in Contexts A and B. Similar to the first test, RE inactivation reduced responding only in Context A. The second experiment followed an identical procedure, except that guide cannulae were bilaterally implanted into the MD. MD inactivation had no effect on responding in either context. Overall, these results suggest that the RE (but not the MD) may work with the PL to support the effects of context on operant responding.

Poster 18

Primary Tissue Derived Brain Organoids Designed for Implantable Cellular Biopharmacies.

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Circadian rhythms are approximately 24-hour endogenous oscillations that function to maintain several physiological processes, ranging from metabolism to immune response to hormone regulation. A misaligned circadian rhythm may desynchronize these processes throughout the body, leading to poor sleep quality and an increased risk for the development of neurologic, metabolic, and/or immune disorders. Melatonin, a hormone produced primarily in the pineal gland, acts to entrain circadian rhythms, and it therefore has significant therapeutic potential for circadian-related disorders. However, exogenous melatonin treatments are limited, as bolus doses do not accurately simulate the natural production of melatonin by the pineal gland, which is tightly regulated by signals to and from the suprachiasmatic nucleus. To overcome this issue, we have developed a cell-based therapeutic system that offers improved timing and dosing control of melatonin. Compared to traditional systems, our system uses novel pineal organoids that enhance production capacity and more accurately model pineal function. For example, the unique composition of the organoids facilitates electrical activation within the device while promoting network maturation, allowing for fine-tuned melatonin production. Overall, this implantable cellular biopharmacy may be a valuable treatment option for sleep disorders, jet lag, and circadian misalignment.

Poster 19

Exploring the Impacts of Genetic Background on Anxiety-like Behaviors at the Baseline Level

Lydia Vadnal, Susan Campbell, Beatrice Girard, & Margaret Vizzard

Anxiety disorders are the most common mental health disorders worldwide, but the underlying factors, causes, and biological mechanisms of these disorders are not well understood. The use of animal models and behavioral tests, specifically the Open Field Test, to investigate anxiety disorders is very important to provide insight into anxiety disorders in humans and help uncover the arcane factors and mechanisms of anxiety disorders. The primary purpose of this research was to explore the impact of genetic background on anxiety-like behaviors in mice at the baseline level, using the Open Field Arena. It was hypothesized that mice with genetic susceptibility to anxiety (B6.Cg-Kit$^{W\text{-}sh}$) would show increased anxiety-like behaviors in the Open Field Arena, characterized by reduced exploratory behavior, and diminished locomotor activity, compared to their wildtype background strain, C57BL/6, while mice with lower anxiety levels (TrpV4) would show decreased anxiety-like behaviors in the Open Field Arena, characterized by increased exploratory behavior and locomotor activity, compared to their wildtype background strain, C57BL/6. The Open Field Arena method was used to collect all data necessary; the locomotor activity, anxiety-related time, and exploratory behavior of B6.Cg-Kit$^{W\text{-}sh}$, TrpV4, and C57BL/6 mice. All data was graphed and analyzed using ANY-Maze software and GraphPad Prism 10. Using the described methods it is shown that both B6.Cg-Kit$^{W\text{-}sh}$ and TrpV4 mice exhibit more baseline anxiety-like behaviors compared to their wildtype background strain, C57BL/6, implying that these strains have a genetic susceptibility to anxiety. The data supported the hypothesis that B6.Cg-Kit$^{W\text{-}sh}$ mice show increased anxiety-like behaviors at the baseline level in the Open Field Arena but did not support the hypothesis that TrpV4 mice show reduced anxiety-like behaviors at the baseline level in the Open Field Arena. While both strains showed increased anxiety-like behaviors when compared to the control C57BL/6 mice, the TrpV4 mice showed much greater anxiety-like behaviors than the B6.Cg-Kit$^{W\text{-}sh}$ mice. The described protocol and resulting data are useful to assess anxiety, test different pharmacological compounds, and investigate the pathogenesis of anxiety disorders. This study utilizes historical approaches for this type of research but could allow for the development of improved therapeutics for anxiety, especially for those with a genetic predisposition.

Poster 20

Tryptophan-producing bacteria modulate 5-HT signaling in the gut and the brain

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OBJECTIVE: There is a high degree of comorbidity between gastrointestinal diseases and psychopathologies, underscoring the need for a better understanding of the bidirectional communication between the gut and the brain. The gut microbiota is an important player in a wide array of functions, ranging from intestinal health to brain neurochemistry and behavior. Exactly how the effects of the gut microbiota are mediated in the brain remain to be elucidated. Serotonin, made from the amino acid tryptophan (Trp), is a key central transmitter regulating behavior. Here we asked whether enhancing the bacterial production of Trp could regulate central 5-HT signaling.

METHODS: *Bacillus* (*B.) subtilis,* is known to express Trp synthase and for its ability to generate Trp. Male C57BL6 mice received an oral gavage of *B. subtilis strain R0179* (10^9 CFUs daily) or vehicle (PBS) for one week. On day 7, blood samples and tissues from the brainstem and hippocampus were collected. Levels of Trp, 5-HT, and 5-hydroxyindoleacetic acid (5-HIAA), the breakdown product of 5-HT, were measured using high performance liquid chromatography.

RESULTS: After one week, a 28.4% increase of Trp levels was observed in blood samples from mice treated with *B. subtilis R0179* (n=9) when compared to PBS-treated mice (n=8). Interestingly, a marked decrease in 5-HT (R0179: 0.74 vs PBS: 0.48 mmole gm^{-1} tissue; p=0.0005) and 5-HIAA (R0179: 1.07 vs PBS: 0.54 mmole gm^{-1} tissue; p=0.0085) were measured in brainstem tissue (n=6) of mice receiving the Trp-synthesizing bacteria compared to control mice. However, in the hippocampus, no changes in 5-HT but lower levels of 5-HIAA were found compared to control (R0179: 20.82 vs PBS: 14.10 mmole gm^{-1} tissue; p=0.0283).

CONCLUSIONS: Trp-synthesizing bacteria in the gut increase blood levels of Trp and differentially modulate 5-HT signaling in the brain in unexpected ways (reducing 5-HT levels). It is possible that the effect on the serotonin signaling pathway observed after one week of treatment with *B. subtilis R0179* represents acute changes and longer time points will need to be studied. The mechanism underlying this modulation of central 5-HT signaling remains to be determined.

Poster 21

Mechanisms of EAE modulation by intestinal colonization with *Akkermansia muciniphila*

Daniel Peipert, MS, Theresa Montgomery, PhD, Dimitry N. Krementsov, PhD,

Multiple Sclerosis (MS) is autoimmune disease of the central nervous system (CNS) in which myelin-reactive immune infiltration contributes to neuronal, neurodegeneration, and subsequent disability. Various studies have documented elevated abundance of the gut bacteria *Akkermansia muciniphila* among patients with MS and animal models of MS compared to healthy control subjects, insinuating it may be a risk factor for disease. However, *A. muciniphila* is widely considered beneficial in various other pathological contexts, and recent studies suggest that *A. muciniphila* may be associated with reduced disability score in MS. Short-chain fatty acids (SCFAs), a category of bacterial metabolites with immune consequences for the host, are modulated by *A. muciniphila* and represent a potential pathway in which *A. muciniphila* may modulate MS. To better understand the role of *A. muciniphila* in MS, we have generated two distinct microbiome models within C57BL/6J mice with and without *A. muciniphila*, providing a variety of ecological contexts in which *A. muciniphila* may behave differently. Using experimental autoimmune encephalomyelitis (EAE) to model MS, we assessed the impact of *A. muciniphila* colonization on disease severity across microbiome models and identified a microbiome in which the addition *A. muciniphila* leads to increased disease severity. Exacerbated EAE severity in *A. muciniphila*-colonized mice was associated with increased Th17 responses and an increased frequency of CNS-infiltrating immune cells. We performed 16S sequencing of fecal samples to identify differences in the gut microbiome by *A. muciniphila* colonization. We observed a reduction of gut Clostridia, key producers of the SCFA butyrate, concomitant with *A. muciniphila* colonization in our microbiome model in which *A. muciniphila* exacerbates EAE. Likewise, analysis of inferred functional pathways relevant to SCFA metabolism highlighted 6 pathways related to reduced butyrate production. Taken together, our data contribute to the complex role of *A. muciniphila* on the severity of CNS autoimmunity.

Poster 22

Pilots of Western Diet and Binge Drinking Effects on the Mouse Brain

Jake Grenon, Ihsan Shawki Akili, R. Chris Skinner

The Western diet is characterized by high amounts of saturated fat, omega 6 fatty acids, simple carbs, and low amounts of fiber. Formerly a diet only associated with developed, Western countries, the Western diet is currently spreading around the world due to the increasing availability of cheap, processed, and calorically dense foods. Additionally, the behavior of binge drinking has become an increasingly more popular form of alcohol intake, especially among young adults. Western Diet alone is linked to neurodegenerative diseases and negative brain health outcomes while binge drinking, and alcoholism can cause neurodegeneration without any nutritional deficiency. Yet, the combination of these two together, commonly seen amongst college students, and their impacts on the brain are not understood. Here, sixty-four 8-week-old C57BL/6J mice (6-10 mice/group) were assigned to one of eight groups: male/female AIN, AIN+Binge, Western, or Western Binge where Western diet mice were provided diet and water *ad libitum* with binge drinking mice receiving a 20% w/v ethanol solution 3 days/week for 6 hours/day to simulate binge drinking. Enzymatic assays were used to measure brain health following 12 weeks of treatment. Results are forthcoming but preliminary data indicates that binge drinking has no further detriment on brain health in the presence of western diet. This research is imperative so that we can make better choices for long term brain health outcomes.

Poster 23

The Impact of Estrous Stage on Behavioral Consequences of Traumatic Brain Injury: A Pilot Study

Anna Lyndaker, B.A., Kathryn Bates, B.S., Olivia MacLean, B.S., Krista DiSano, PhD., Paul Holtzheimer, MD, & Crystal Noller, PhD

Traumatic brain injury (TBI) is a significant cause of mortality and disability globally, yet the impact of sex-specific factors on injury progression is not fully understood. Overall, women report worse outcomes after mild TBI, such as more severe post-concussion syndrome, depression, anxiety, and post-traumatic stress disorder (PTSD) symptoms relative to men. Conversely, women demonstrate reduced TBI-related mortality and improved recovery from moderate or severe TBI. Rodent studies mimic these findings, with females demonstrating improved recovery in motor function, spatial memory, and anxiety- and depressive-like behaviors following TBI compared to injured males. However, these sex differences may be masked or misrepresented if hormone fluctuations during estrous phases are not considered. Research suggests that rodents injured during high estrogen phases have better neurologic function after neurotrauma, suggesting that estrogen is neuroprotective. One human study also found that women injured during their follicular (high estrogen) phase reported better post-injury symptoms than women in their luteal (low estrogen) phase. Using a mouse model of TBI, the current study was designed to determine how estrous phase influenced behavioral outcomes in three domains of impairment: cognition, anxiety, and depression.

Poster 24

Kathryn Bates

Poster 25

PI3K Is Essential for Maintaining Sema6A Induced Eye Vesicle Cohesion in the DevelopingZebrafish

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Semaphorin6A (Sema6A) is a transmembrane protein that plays important roles in development, including aspects of cell migration, adhesion, proliferation, and differentiation (Alto & Terman, 2017). Plexins are the main functional receptors of Semaphorins. When a Semaphorin binds to Plexin this initiates forward signaling through the intracellular domain of Plexin, which has been widely studied and shown to have a necessary role in zebrafish eye development (Ebert et al., 2014). Published data from our lab has shown that Sema6A functions to maintain cohesion in the eye field of zebrafish, however it is unknown which downstream signaling pathway(s) Sema6A/PlexinA2 are using to regulate eye cohesion (St. Clair et al., 2018 & 2019). Cultured eyefield explants show loss of integrity which is prevented by adding Sema6A. Using pharmacological inhibitors and conditioned media, we have identified the necessity of specific signaling pathways involved in the maintenance of eyefield cohesion.

Poster 26

Investigating the Role of DCBLD2 in Zebrafish Visual System Development and Function

Lila Rose Tomlinson, Bryan Ballif, Alicia Ebert Department of Biology, University of Vermont

Our lab investigates the signaling pathways responsible for patterning the developing retina. As we uncover these pathways, we are always left wondering how the embryo can see with these retinal disruptions. An optokinetic response chamber is the most common method for quantitatively assessing the visual acuity of zebrafish (*Danio rerio*). For my summer research project, I built an effective and easy-to-use optokinetic response chamber used to observe eye movement in response to visual stimuli of the entire visual field. The chamber uses rotating black and white stripes as visual stimuli with varying cycles per degree (cpd), or thickness of the stripes. Zebrafish embryos will track the stripes, and their eyes will rotate in the socket to follow them. This response can be visualized and recorded easily from above. I have worked towards creating a comprehensive protocol for its use consisting of testing wild-type zebrafish embryos. Various factors have to be considered such as embryo age, light intensity, cpd, speed, and how the embryos are held in place. After various preliminary rounds of testing, I determined the best mode of testing to be the consistent use of a single cpd and increasing the rotations per minute until the visual tracking ceases. I am currently using this chamber to test zebrafish embryos lacking the orphan receptor DCBLD2. These mutants have normal retinas until 8 days post-fertilization (dpf) where the back of the eye blows out and retinal neurons spill into the brain. I have not observed a significant difference in visual acuity between wildtype and mutant embryos at 4 and 5 dpf, however, I will continue to test older embryos up to when the phenotype becomes apparent at 8 dpf.

Poster 27

Abl-mediated phosphorylation of conserved Semaphorin6A tyrosine residues is necessary for growth cone collapse and zebrafish retinal integrity

Collin M. MacLeod, Caroline M. Dumas, Gillian G. Berglund, Taylor E. Bean, Bryan A. Ballif, and Alicia M. Ebert

Nervous system development is guided by communication and interpretation of many signaling molecules. One such signaling pathway necessary for nervous system development is Semaphorin (Sema) and Plexin (Plxn) signaling. Sema6A and PlxnA2 function as a bidirectional signaling pair, meaning that both proteins can act as a ligand or receptor. Sema6A and PlxnA2 are implicated in neuronal growth and migration, and function as a repulsive axon guidance cue, which leads to growth cone collapse and retraction upon binding with their respective ligand pair. We have previously shown that genetic disruptions in zebrafish of either Sema6A or PlxnA2 signaling leads to significant impairments in eye size and retinal development. While much of the research to date focuses on forward signaling through Plxn, we have shown a critical role for Sema6A reverse signaling in zebrafish. In rescue experiments using a truncated Sema6A lacking its intracellular domain, we showed reverse signaling is not necessary for eye size or retinal lamination, however, it is necessary for retinal integrity, Müller glia development, and cell survival. We are now exploring potential downstream signaling pathways, including Abl kinase-mediated Sema6A phosphorylation in both mammalian neuronal cell culture and zebrafish retinal development. We have identified by mass spectrometry conserved tyrosine residues on the Sema6A intracellular domain which are phosphorylated by Abl. In rescue experiments using constructs with these tyrosines mutated to phenylalanines, we revealed these residues are necessary for neuronal growth cone collapse and zebrafish retinal integrity.

Poster 28

Dopamine and insulin receptor signaling in sweet taste cells influence protein and sugar sensing in *Drosophila melanogaster*

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Internal state consists of a variety of factors and metabolic pathways which are responsible for modulating feeding behavior. Hunger is an essential cue in animals to trigger this behavior, and dopamine has previously been established to increase sweet taste sensitivity in a fasted state in the fruit fly, Drosophila melanogaster (Inagaki et al, 2012). This data has been replicated and further expanded upon to include amino acid taste sensitivity in sweet gustatory receptor neurons (GRNs). Drosophila insulin like peptides (DILPs) are ligands to the tyrosine kinase insulin receptor (InR), which is a conserved insulin receptor with humans. DILP levels fluctuate depending on satiation, and their exact role at the primary sensory level is unknown. We use the Gal4-UAS system to express RNAi directed against the dopamine receptor (DopEcR) or express a dominant negative form of the InR in the primary sweet taste GRNs, using a Gr64f-Gal4 driver. Using the proboscis extension response (PER), where a fly is mounted in place and the GRNs stimulated with a solution, we show that dopamine is responsible for increase sensitivity to both sugar and amino acids whereas insulin is more involved in suppressing sugar sensitivity. To examine nutrient consumption in freely- behaving flies, we used the Fly Liquid-Food Interaction Counter (FLIC). FLIC data shows that expressing InR[DN] leads to an increased number of feeding events for amino acids, but interestingly, a decrease in the length of consumption. Conversely, for sucrose solutions the number of feeding events is not impacted, but expressing InR[DN] leads to an increase in feeding bout duration. FLIC data for DopEcR RNAi lines is complementary to PER, showing a suppression in food interactions and number of feeding events across nutrients while increasing length of feeding bouts. This data suggests that dopamine is essential for eliciting feeding behavior for amino acids and sucrose in Drosophila, while insulin receptors have a nutrient-dependent role.

Poster 29

Impact of Surgical Bladder Catheter Implantation on Voiding Function in Male and Female Mice

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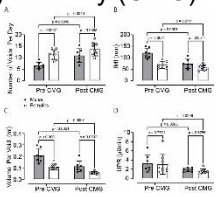
INTRODUCTION AND OBJECTIVES: Lower urinary tract (LUT) disorders significantly impact public health, affecting millions in the United States. Developing effective treatments requires suitable animal models for studying normal and pathological urinary tract function. In mice, LUT function is commonly assessed through invasive urodynamics and non-invasive voiding assays. Invasive urodynamics (cystometry; CMG) involve surgical bladder catheter implantation, which may affect bladder function. Non-invasive voiding assays, such as UroVoid and Void Spot Assays on Paper, assess normal spontaneous voiding patterns while animals are in a home-cage environment. This study aims to compare non-invasive voiding behavior before and after CMG to determine how catheter implantation impacts normal bladder function in male and female mice.

METHODS: Sixteen mice (n=7 males and n=9 females) underwent a four-phase study: (1) 4-day non-invasive voiding assay using UroVoid; (2) surgical bladder catheter implantation under isoflurane anesthesia (1-3%) followed by a 4-day recovery period with daily analgesic administration (carprofen, 5 mg/kg s.c.); (3) CMG for urodynamic recording; and (4) a final 4-day non-invasive UroVoid assessment. All statistical differences were assessed using two-way repeated-measures ANOVA with Fisher's LSD for multiple comparison and statistical significance was designated at $P < 0.05$. All statistical analyses were conducted using Prism.

RESULTS: Following bladder catheter implantation, mice demonstrated notable alterations in voiding behavior. Both sexes showed a significant increase in daily void frequency (Figure 1A). Average void volumes, indicative of bladder capacity, significantly ($p < 0.0001$) decreased by 46%, from 0.151 ± 0.067 mL to 0.081 ± 0.042 mL, with comparable changes observed across sexes (Figure 1C). Changes in intermicturition intervals (IMI) and urine production rates (UPR) were also observed, with the degree of change varying between sexes (Figure 1B, D).

CONCLUSION: Although CMG has been the gold standard for assessing bladder urodynamics, it is crucial to recognize the functional alterations induced by this procedure, as demonstrated by significant changes in bladder function observed in non-invasive voiding assays. These procedural impacts must be accounted for when translating findings from preclinical mouse models to therapeutic strategies for LUT disorders in clinical settings.

Figure 1. Spontaneous voiding behavior assessed using UroVoid in male and female mice before and after surgical catheter implantation for cystometry (CMG)



Poster 30

Gallbladder smooth muscle dysfunction occurs independently of inflammation in gallstone disease

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OBJECTIVE: There is a link between gallbladder dysmotility and the development of cholecystitis in gallbladder disease, but the relationship between compromised muscle function and inflammation are not clearly understood. Using a mouse model of gallstone disease, we challenged the concept that inflammation leads to gallbladder smooth muscle (GBSM) dysfunction, by providing evidence that GBSM dysfunction is an early step in the development of the disease and precedes gallbladder inflammation. Here we investigate the mechanisms.

METHODS: We performed histopathology, a mouse immune panel (Luminex Assay; R&D Systems), and calcium imaging on gallbladders from mice fed a lithogenic or control diet for 1-8 weeks, with or without COX-2 inhibitor meloxicam (0.3 mg/kg/day orally) treatment.

RESULTS: In mice fed a normal diet, GBSM cells exhibit rhythmic, synchronized Ca^{2+} flashes which correspond to action potentials. In mice fed a lithogenic diet, disrupted GBSM flash activity was observed as early as 1 week after the start of the diet and by 4 weeks, regular Ca^{2+} flashes were rarely observed. No histopathological features of gallstone disease which include gallbladder wall thickening secondary to muscle hypertrophy, epithelial hyperplasia and inflammation were observed in control mice. No changes were observed in wall thickness and lymphocytes were rarely observed after one to two weeks on the lithogenic diet. Epithelial hyperplasia, muscularis hypertrophy and expansion of the lamina propria by some inflammatory cells only became apparent at 4 weeks. No clear evidence of inflammatory cytokines were observed in the gallbladders of mice on the lithogenic diet for 1-4 weeks. The gallbladders of mice treated with the COX-2 inhibitor, while being fed the lithogenic diet for 4-8 weeks have dramatically reduced inflammation (less lymphocytic infiltration and muscle hyperplasia) compared to mice on the lithogenic diet without treatment. However, all mice fed the lithogenic diet, with or without COX-2 inhibitor treatment, had cholesterol crystals in their gallbladder and presented abnormal GBSM activity characterized by bursts of action potentials separated by quiescent periods.

CONCLUSION: These results suggest that changes in GBSM function are independent of inflammation. This represents a paradigm shift in the field and creates the potential for earlier interventions.