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Platform Talks and Poster Abstracts

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Platform Talk 1

Brain-derived neurotrophic factor: a novel regulator of cardiovascular function

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Brain-derived neurotrophic factor (BDNF) is a member of the neurotrophin family and has a key role in regulating neuronal development and survival. In addition, increasing evidence indicates that neuronal activity-dependent production and release of BDNF provokes both short-term and long-term changes in synaptic function and that BDNF may also act as a neurotransmitter. The paraventricular nucleus of the hypothalamus (PVN) plays a central role in neural control of cardiovascular function, and BDNF synthesis in the PVN has been shown to increase in response to hypertensive stimuli including stress and hyperosmolarity. However, it is unclear whether BDNF, acting within the PVN, contributes to elevations in blood pressure. Using radiotelemetric blood pressure monitoring in Sprague-Dawley rats, we have established that 1) viral vector-mediated overexpression of BDNF in the PVN induces significant increases in mean arterial pressure; heart rate and indices of sympathetic activity; 2) acute microinjection of BDNF into the PVN elicits rapid elevations in blood pressure and heart rate; 3) both acute and long-term effects of BDNF are mediated at least in part by changes in the brain renin angiotensin system; 4) chronically elevated BDNF in the PVN upregulates catecholamine biosynthesizing enzymes in certain nuclei of the brainstem. These results identify BDNF as a significant regulator of cardiovascular function that may play an important role in mediating stress-induced hypertensive responses.

Platform Talk 2

Mucosal 5-HT4 Receptors as a Novel Therapeutic Target in Colitis

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We have previously shown that 5-HT4 receptors are expressed in colonic epithelium, and that 5-HT4 agonists produce physiological responses such as mucus secretion from goblet cells, chloride secretion from enterocytes, and serotonin release from enterochromaffin cells. These responses may have protective and/or recuperative actions in the colon in pathological conditions such as colitis. To test this hypothesis, we induced colitis in CD-1 mice using 4% DSS (w/v) in drinking water or a single enema of TNBS (7.5mg/mL in 50% ethanol). The mice were treated with the 5-HT4 receptor agonist, Tegaserod (1mg/kg), Tegaserod plus the 5-HT4 antagonist, GR113808, or vehicle. Animals were treated during (days 1-7) or following (days 7-15) the development of colitis. To test the functional effect of this treatment on propulsive motility, TNBS treated guinea pigs were administered tegaserod on days 1-7, and distal colon motility was evaluated. Disease Activity Index (DAI) and histological damage scores were used to evaluate the extent of inflammation. Epithelial 5-HT4 receptor activation reduced the severity of colitis in the 7d DSS model compared to vehicle treated inflamed animals as measured by DAI ($p<0.05$) and H&E scores ($p<0.001$), and this effect was inhibited by 5-HT4 receptor blockade. When animals were treated after colitis was established, recovery from colitis was accelerated by 5-HT4 receptor activation. Furthermore, propulsive motility, which is dramatically disrupted in TNBS colitis was significantly improved by 5-HT4 agonist treatment. Taken together, these data suggest that activation of mucosal 5-HT4 receptors reduces the development of, and speeds recovery from, inflammation.

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Platform Talk 3

Studying the Role of the TRPV3 Channel in Mouse Urinary Bladder Function and Sensation

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Overactive bladder (OAB) is a significant health problem in the United States. Individuals with OAB suffer from symptoms such as a sudden strong urge to urinate which is difficult to differ, and in some cases it is associated with urine leakage, as well as the frequent need to urinate during the day and at night. None of the current therapeutic modalities for the treatment of OAB are universally effective and all are associated with significant side effects.

In animal models, various transient receptor potential (TRP) cation channels were shown to be involved in bladder functioning, mechanosensory transduction, and nociception. Pharmacological targeting of these channels however failed to result in clinical improvement in OAB patients. This failure has led to speculation that an additional TRP channel is involved. TRPV3 has previously been identified as having a role in sensory signaling in the peripheral and central nervous systems regulating various organs and systems. The goal of this study was to evaluate the role of TRPV3 in bladder function and sensory signaling. We studied its expression in sensory neurons in the L6 and S1 dorsal root ganglia (DRG) in healthy mice and in a mouse model of OAB and conducted functional studies to compare the lower urinary function between the healthy wild type and TRPV3-knock out (KO) mice. We compared expression of TRPV3 in DRG from mice with surgically-induced partial bladder outflow obstruction (pBOO) to those from healthy control mice. TRPV3-KO mice served as negative controls. Using immunohistochemistry and RT-PCR we conducted a qualitative and quantitative assessment of TRPV3. Additionally, behavioral studies evaluating the micturition frequency as well as cystometrography were used to compare the phenotypic presentation between wild type and TRPV3-KO mice.

Compared with age-matched controls, pBOO mice exhibited two-fold increase in TRPV3 mRNA expression. Cross-sections of DRG showed an increase in the number of TRPV3-positive neurons (n=8). Compared to healthy controls, the voiding frequency (n=12/per group) in TRPV3-KO animals, was reduced by 40% and CMG recordings showed increase in the intermicturition interval (n=6/group).

These results support our hypothesis that TRPV3 exerts a role in bladder function and sensation.

Platform Talk 4

Gastrointestinal Dysmotility in a Mouse Model of Multiple Sclerosis

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Multiple Sclerosis (MS) patients often experience constipation, but the etiology of this symptom is unknown. We tested the hypothesis that mice with experimental autoimmune encephalomyelitis (EAE) would exhibit delayed gastrointestinal (GI) transit that is mediated by antibodies directed against targets in enteric ganglia. EAE was induced in C57BL/6J mice by injection of complete Freund's adjuvant (CFA) and myelin oligodendrocyte glycoprotein. After development of somatic motor symptoms, small intestinal transit was measured by calculating the leading edge (LE) and geometric center (GC) 20 minutes after oral gavage of rhodamine dextran. Whole GI transit time was determined by the latency of dye to appear in fecal pellets after oral gavage of carmine red. Targets of antisera were evaluated by immunohistochemical staining of guinea pig intestine myenteric plexus with EAE and control plasma. Small intestinal transit was significantly slower in EAE mice than in healthy controls (LE, $p < 0.02$; GC, $p < 0.01$), and was not altered in CFA controls. The rate of whole GI transit was significantly longer in EAE mice versus healthy controls ($p < 0.05$). Immunoreactivity against myenteric ganglia was more intense when stained with plasma from EAE mice versus plasma from healthy control mice, with immunostained structures including neurons and nerve processes. Inconclusion, EAE causes delayed small intestinal and whole GI transit time compared to healthy control mice, which may represent the bowel dysmotility exhibited by MS patients. These symptoms maybe explained by the presence of antibodies in the blood of EAE mice that target cells of the enteric ganglia.

Platform Talk 5

The contribution(s) of transforming growth factor-beta to bladder afferent nerve hyperexcitability with cyclophosphamide-induced cystitis

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Bladder Pain Syndrome (BPS)/Interstitial Cystitis (IC) is a major unresolved health concern in the United States with a considerable economic burden in lost productivity and health care. We hypothesize that the pleiotropic protein, transforming growth factor-beta (TGF-β), and its cognate receptors contribute to afferent nerve hyperexcitability that may facilitate bladder hyperreflexia in an experimental cystitis model of BPS/IC. To determine the role of TGF-β in bladder afferent nerve hyperexcitability we used an ex vivo bladder-pelvic nerve preparation in control and CYP-treated tissues. Intravesical instillation of recombinant TGF-β in control tissues significantly increased mean afferent nerve frequency (imp/sec) at various bladder distentions. Forty-eight hours following CYP-induced cystitis, mean afferent nerve frequency (imp/sec) significantly increased relative to control tissues. The intravesical instillation of a TGF-β type I receptor inhibitor significantly decreased the enhanced mean afferent nerve frequency (imp/sec) in CYP-treated tissues. Given its spatiotemporal regulation in the urinary bladder inflammatory response, these results suggest TGF-β may, in part, play a role in the development of lower urinary tract symptoms in BPS/IC by contributing to afferent nerve hyperexcitability and peripheral and central sensitization. Manipulation of this signaling pathway following bladder inflammation may provide a novel therapeutic opportunity to alleviate lower urinary tract symptoms.

Platform Talk 7

Regulation of Cerebellar Kv1.2 by PKM-ζ and its Implication for Learning and Memory

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PKC-ζ and its N-terminal truncated form PKM-ζ, have long been implicated in the regulation of hippocampal AMPA receptors in a cellular correlate of learning, long term potentiation (LTP) (Ling et al., 2002; Yao et al., 2008). Yet little is known about the function of PKM-ζ in the cerebellum. Both PKC-ζ and PKM-ζ are highly expressed in the cerebellum (Oster et al., 2004) and our lab has shown that endocytosis of Kv1.2 in Basket Cells and Purkinje Cells can enhance the acquisition of eye-blink conditioning (EBC) in rats, a form of cerebellar learning (Williams et al., 2012). Having been implicated in the regulation of Kv1.2's β subunit, we hypothesized that PKC-ζ and PKM-ζ may have regulatory effects on Kv1.2, and that this may have implications for cerebellar function. We have shown for the first time that both PKC-ζ and PKM-ζ can alter Kv1.2 surface expression in HEK 293 cells. Furthermore we have shown that inhibition of PKM-ζ/PKC-ζwith ZIP (Zeta-inhibitory peptide) can significantly disrupt eye-blink conditioning in rats.

Platform Talk 8

Reducing Relapse by Presenting a Reinforcer Associated with Behavioral Inhibition

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Human behavior accounts for a wide array of negative health outcomes. While there are behavioral treatments that are effective at reducing maladaptive behaviors like cigarette-smoking, drug-taking, and overeating in the short run, they often leave the behavior susceptible to relapse. Operant conditioning is an important model of voluntary behavior that can be studied in animals and used to understand conditions that encourage behavioral relapse following treatment. In this method, subjects are free to respond at any time (e.g., by lever pressing) to earn a reinforer (e.g., a food pellet). Operant behavior can be suppressed or inhibited by a process known as extinction, in which the organism learns that the behavior no longer produces the reinforcer. Although extinction is part of many behavioral treatments, behavior that has been inhibited or "extinguished" this way is prone to return or relapse. In one example, "resurgence," an extinguished behavior can return when a new behavior that was reinforced to replace it is no longer reinforced. In a second example, "renewal," extinguished behavior returns when it is tested in a new context or situation. Both examples of relapse demonstrate that the context in which extinction occurs controls inhibition of the voluntary behavior. In renewal, the context is the physical background, whereas in resurgence, the "context" is theoretically created by the reinforcers earned by performing the replacement behavior. Two experiments with rats therefore tested whether reinforcer presentations could control behavioral inhibition and thus be used to prevent relapse. In both experiments, presentations of unique alternative reinforcers that had been associated with extinction suppressed behavior as it relapsed when the reinforcement contingencies or context were changed (resurgence and renewal, respectively). The effects of the physical context and reinforcer "context" were additive. The results have implications for understanding and preventing relapse after incentivized treatments to remove unhealthy behaviors. For example, they continue to suggest that one way to potentially reduce relapse is to encourage generalization from the treatment context to other contexts.

Platform Talk 9

The role of ACTH in improving cognitive outcomes in pediatric epilepsy

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Children with epilepsy often present with pervasive cognitive deficits and behavioral comorbidities including working memory impairments, ADHD and autism spectrum disorder. These non-seizure characteristics are severely detrimental to overall quality of life for patients and their caregivers. Efforts at improving these deficits with treatment of seizures and epileptic discharges have only had modest impact, and very few treatment strategies are currently available to treat cognitive deficits themselves. Therefore, it is important to expand our search for treatment strategies beyond drugs that are only effective in treating seizures to drugs that may alter underlying disease pathways or pathways that are important for cognition, thereby improving outcome. We have preliminary data suggesting that ACTH prevents cognitive deficits in two early life epileptiform activity (EA) models: one with overt generalized seizures (ELS) and one with only focal EA in the prefrontal cortex. In the ELS model, ACTH is able to prevent deficits in fear extinction; in the EA model, ACTH is able to prevent an attention deficit seen in these animals. These results suggest that ACTH may be effective in preventing deficits in many different cognitive domains, domains that are often affected in patients with pediatric epilepsy. Furthermore, ACTH exerts this effect without altering either latency to or duration of seizure in the ELS model, or any of the spiking characteristics in the EA model. Future studies will test the hypothesis that the action of ACTH on central melanocortin receptors is the mechanism by which the drug acts to prevent deleterious cognitive outcomes.

Platform Talk 10

Astrocyte calcium signaling drives inversion of neurovascular coupling after subarachnoid hemorrhage

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Increased local cerebral blood flow (CBF) and associated increases in oxygen delivery are crucial for maintaining neuronal function and survival in active brain regions. The process by which local CBF is dynamically regulated to meet the ongoing metabolic demand of active neurons is called functional hyperemia or neurovascular coupling (NVC). Briefly, NVC in the healthy brain involves: 1) increased synaptic transmission, 2) a propagating wave of Ca^{2+} in associated astrocytes that terminates in the perivascular endfeet, 3) Ca^{2+}-dependent release of vasodilatory substances (e.g. K^+ efflux via large conductance Ca^{2+}-activated K^+ [BK] channels) from the endfeet onto the underlying arteriole and 4) arteriolar dilation and increased local CBF. Recently, inversion of NVC from vasodilation to vasoconstriction was demonstrated in brain slices obtained from subarachnoid hemorrhage (SAH) model rats (Koide et al. PNAS 109: E1387-E1395, 2012). This pathological response, which could restrict blood flow to active brain regions, coincided with the increased amplitude of spontaneous Ca^{2+} events in astrocytic endfeet. Here, our goal was to provide evidence of a causal link between these two phenomena. The inversion of NVC and high amplitude spontaneous endfoot Ca^{2+} events were first observed in brain slices (≈ 70 % of slices) from animals 24 hr post-SAH. At 48 hrs and 96 hrs post-SAH, nearly all brain slices exhibited inversion of NVC and high amplitude spontaneous Ca^{2+} events. Additional studies were performed measuring both NVC and spontaneous Ca^{2+} activity in continuous recordings from brain slices using 24 hr SAH rats, where only EFS-induced dilation or constriction was observed in a given brain slice. All brain slices exhibiting EFS-induced vasoconstriction were accompanied by high-amplitude spontaneous Ca^{2+} events in the surrounding endfeet, whereas only low-amplitude events were observed around vessels that dilated. Further, to mimic EFS-induced K^+ release through endfoot BK channels, we raised the extracellular K^+ concentration ($[K^+]o$) from 3 mM to 10 mM in the brain slice superfusate. As expected, this modest elevation of $[K^+]o$ caused vasodilation in brain slices from control animals, whereas it caused constriction after SAH, consistent with SAH-induced inversion of NVC. Interestingly, pharmacologic depletion of intracellular Ca^{2+} stores, which abolished all spontaneous Ca^{2+} activity in the endfeet, restored arteriolar dilation to a modest elevation of $[K^+]o$ in brain slices from SAH animals. Together, our data demonstrate a key role for the increased amplitude of spontaneous Ca^{2+} events in astrocytic endfeet in causing inversion of NVC after SAH.

Platform Talk 11

A Novel Role for Collapsin Response Mediator Protein 2 in the Development of the Vertebrate Visual System

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The development of a functional nervous system is dependent on neurons extending their axons to form appropriate connections with their targets. This migratory process is dependent on the growth cone of the neuron responding to repulsive and attractive signals in the environment. Repulsive guidance cues, such as Semaphorins, induce the growth cone to collapse and change direction via interaction with Plexin and/or Neuropilin receptors. Collapsin Response Mediator Proteins (CRMPs) are a family of microtubule-associated proteins that are regulated by phosphorylation through a signaling cascade initiated by Semaphorins. CRMPs have important functions in the proper positioning and lamination of cortical neurons. However, the function of CRMPs is not yet understood in laminated, non-cortical areas of the brain, such as the retina. The objective of this study is to determine the function of CRMP2 in the development of the retina and optic tract, using zebrafish as a model. First, we show using in situ hybridization, that crmp2 mRNA is expressed in the retinal ganglion cells, the output cell of the retina. Next we show that decreasing crmp2 expression results in a smaller retinal ganglion cell layer as well as impaired optic tract formation and retinal lamination. These data suggest a novel role for CRMP2 in the appropriate lamination of the retina as well as the development of the optic tract.

Platform Talk 12

Activity-dependent serotonergic excitation of callosal-projection neurons in the mouse prefrontal cortex

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Serotonin (5-HT) is an important neuromodulator within the prefrontal cortex (PFC), yet many mysteries remain regarding how 5-HT regulates the output of cortical circuits. We've recently shown that serotonergic responses in layer 5 pyramidal neurons (L5PNs) in the mouse medial PFC (mPFC) depend on their long-distance axonal projections; corticopontine (CPn) neurons are inhibited by 5-HT via activation of 5-HT_{1A} (1A) receptors, while commissural/callosal (COM) neurons exhibit excitatory responses to 5-HT that are mediated by 5-HT_{2A} (2A) receptors. Among COM neurons, 2A-dependent excitation can occur in isolation (in "COM-excited" neurons), or following brief 1A-mediated inhibition (in "COM-biphasic" neurons). Here we further characterize subpopulation-specific serotonergic signaling in COM and CPn L5PNs in the mouse mPFC. We report that excitation of COM neurons is state-dependent: excitatory serotonergic responses were more robust when 5-HT was paired with suprathreshold somatic depolarization. On the other hand, inhibitory responses to 5-HT in CPn and COM-biphasic neurons were less dependent on membrane potential. In additional experiments, we paired 5-HT with suprathreshold or subthreshold simulated synaptic input generated by somatic current injection. In COM-excited L5PNs, 5-HT increased the number of action potentials (APs) generated by suprathreshold simulated input, but did not induce AP generation, or increase voltage response integrals, when paired with subthreshold simulated synaptic input. Conversely, 5-HT decreased AP number, reduced response integrals, and hyperpolarized CPn and COM-biphasic neurons when paired with simulated synaptic input. Finally, we tested the interaction of 1A and 2A receptors in CPn and COM-excited neurons. After pharmacological blockade of 1A receptors, pairing 5-HT application with somatic depolarization produced moderate 2A-dependent excitation in ~32% of CPn neurons. *Post hoc* analysis revealed that the presence of 2A receptors in CPn L5PNs coincided with a reduced duration of 1A-mediated inhibitory responses in baseline conditions when 5-HT was paired with low, but not high, frequency firing. On the other hand, 1A-mediated responses were never revealed in COM-excited neurons following blockade of 2A receptors. Together, our results suggest that 2A-mediated excitation will be most effective in COM-excited neurons experiencing coincident suprathreshold excitatory drive. This feature of 2A-mediated excitation may provide a mechanism for selective serotonergic amplification of active COM output channels.

Platform Talk 13

Viral strategies to probe autism-associated genes in developmental neurophysiology

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Developing neurons must establish connections of both the right number and strength with appropriate synaptic partners, and tightly regulate their intrinsic excitability. Autism spectrum disorder and epilepsy are neurological disorders that have a high comorbidity, and in a subset of these disorders, altered neuronal excitability is hypothesized to be causative. The dual-specificity phosphatase, Pten, has repeatedly been found to be mutated in a subset of patients having autism with macrocephaly, some of which also have seizures. Correspondingly, in mouse models where Pten is deleted from neurons, behavioral changes reminiscent of autism symptoms and seizures have been reported to emerge. To understand how loss of Pten function effects neuronal development, we have used co-injection of retroviruses which selectively infect newborn granule neurons in the dentate gyrus of the neonatal mouse. These retroviruses either label newborn neurons witha fluorescent protein, or label with a distinct fluorescent protein and delete Pten. By conducting studies on the morphological and electrophysiological differences between control and Pten depleted neurons in the same animal, and between animals at increasing days post infection, we present an integrative analysis of how the structure/function relationship of developing neurons is disturbed in this genetic model of autism with epilepsy. Specifically, we have found that Pten depleted developing neurons establish an excess of excitatory synaptic inputs, that together with deranged intrinsic excitability and cellular morphology, ultimately leads to neuronal hyperactivity.

Platform Talk 14

Structural Dynamics of Tau: Implications for Neurodegenerative Disease

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Tau is a conformationally dynamic microtubule-associated protein expressed at high levels in neurons. It is localized primarily in the axonal compartment where it has been implicated in a number of intracellular functions, including microtubule stabilization, cross-linking between the cytoskeleton and plasma membrane, acting as a scaffold for a variety of signaling molecules, and modulating the axonal transport process. Tau's myriad of functions are likely to be related to its conformationally dynamic structure, but the structure/function relationships within this molecule remain poorly defined. Additionally, Tau is known to be regulated by phosphorylation at numerous Ser/Thr and Tyr sites throughout the molecule, but the effects of phosphorylation on Tau's structural dynamics are also unclear. Previous in vitro work in our lab has shown that Tau interconverts between static and mobile (diffusive) states on the microtubule surface in an isoform and microtubule-lattice specific manner (McVicker et al., (2014) Cytoskeleton 71:184). To further extend these studies into a physiologically relevant environment in which Tau is naturally regulated by phosphorylation, we have used single-molecule imaging to examine the dynamic behavior of fluorescently-labeled Tau on the surface of microtubules within the isolated axoplasm of the squid giant axon. Tau maintains its isoform-specific ability to interconvert between static and diffusive states on the microtubule surface under these conditions. Furthermore, we demonstrate phosphorylation influences Tau's dynamic behavior on the microtubule surface, e.g., inhibition of CDK5 by Roscovitine results in a significant shift in Tau's dynamic equilibrium towards the diffusive state. These studies provide new insight into the role of phosphorylation in regulating Tau's structural dynamics on the microtubule surface and its potential role in the development of neurodegenerative diseases including Alzheimer's and FTLD (Fronto-Temporal Lobe Dementia).

Platform Talk 15

Genome-Wide Mapping of Methamphetamine Sensitivity in Commercially Available Outbred Mice

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Sensitivity to the locomotor activating effects of methamphetamine (MA) shares overlapping neurocircuitry with brain areas associated with reward and may contribute to risk for drug abuse disorders. Individual differences in initial sensitivity to MA are controlled in part by genetic factors; however, identifying genes underlying these differences has proven difficult. We have taken advantage of an extant outbred population that has been maintained using an outbred breeding scheme for more than 100 generations to identify and map narrow quantitative trait loci (QTL) associated with sensitivity to the locomotor stimulant response to MA. Following protocols we have developed in previous work, we phenotyped ~1200 male CFW mice for MA sensitivity. We developed a novel genotyping pipeline based on the "genotype-by-sequencing" approach to generate high-density SNP data (~90,000 SNPs) across the genome in 1161 mice. Next, GWAS was performed using a linear mixed model to account for confounding due to relatedness. Finally, we performed RNASeq on three brain regions (prefrontal cortex, hippocampus, and striatum) from a subset of animals in order to explore the network of correlations that exist between DNA sequence, gene expression values and MA sensitivity. We identified two narrow QTL peaks that reached genome-wide significance, on chromosomes 6 ($p = 9.03 \times 10^{-7}$), and 9 ($p = 1.58 \times 10^{-6}$). In the QTL on chromosome 6, the peak SNP is located in a gene desert, however, the nearest gene to it is Ctnna2, which is a regulator of synaptic plasticity. It has been implicated in excitement seeking, ADHD, and substance use disorders (SUDs) in human GWAS, and therefore is a compelling candidate. The chromosome 9 QTL contained three genes within its region (Rbms3, Cmc1, Azi2). Although these genes have not been associated with SUDs to date, we observed expression QTLs for two of these genes (Rbms3 & Cmc1), making them promising candidates for follow-up studies. Thus, by exploiting the increased recombination frequency in outbred mice, we mapped behavioral and gene expression QTLs with significantly greater precision than previous approaches and can begin to identify plausible biological explanations for how these alleles influence behavior and thereby implicate specific genes.

Platform Talk 16

Alcohol Dependence and Copy Number Variations

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To identify alcohol dependence (AD)-associated copy number variations (CNVs), we carried out a genome-wide CNV study of AD in 6,950 African- and European-Americans, representing one of the largest known AD cohorts. We assayed the samples using the Illumina genotyping arrays of one million probes. After quality control and data analysis, we found that the AD patients contained significantly more CNVs than controls ($P = 1\times10\text{-}23$). The frequencies of one specific CNV on cytogenetic band 22q11.2 were 20% in cases and 10.6% in controls of African-Americans ($P = 8\times10\text{-}6$ and OR (95% CI) = 2.1 (1.63-2.7)) and 23% in cases and 3.8% in controls of European-Americans ($P = 1\times10\text{-}7$ and OR = 7.41 (4.09-13.43)). The meta-analysis showed $P = 4\times10\text{-}15$ and OR = 2.55 (2.02-3.22). The CNV boundaries that we predicted based on our microarray data are well-consistent with those from public sequence dataset. The gene that we identified is within the well-known 22q11.2 deletion region, which has been well-known for associations with numerous AD-related behavioral problems and mental illnesses. In this study, we newly identify in this large deletion region a specific gene is likely a responsible gene for AD. We validated this CNV in randomly-selected samples using qPCR and Long PCR. Additionally, a previous study of rat quantitative trait locus of alcohol consumption maps to a one million base pair region where our identified gene is located in the middle of this linkage region.

Poster 1

Representation of predacity of animal species in the human brain

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A classic studies in the semantic representation of animal categories suggested that predacity – or the perceived aggressiveness or dangerousness of animals – is a central dimension predictor for behavioral judgments of semantic similarity among animal species. In this fMRI, study we locate networks that reflect predacity related neural processing independent of taxonomic class and low-level visual features. Using support-vector machine pattern classification within whole-brain searchlights, we mapped networks that distinguish activation patterns elicited by viewing images of high-predacity animals (e.g., wolves, scorpions) versus low-predacity animals (e.g., rabbits, ladybugs). We controlled for taxonomic class by training and testing across class-based data folds—we trained the classifiers to distinguish between high and low predacity for one class (eg, mammals or reptiles) and tested for generalization to another class (bugs). These analyses yielded predacity-relevant networks in the right superior temporal sulcus (RSTS), left anterior intraparietal sulcus (LIPSa), and calcarine sulcus. We then used clustering to group the predacity-sensitive surface nodes into separate networks based on similarity of local representational spaces. The clustering solution comprised four large clusters: CALC, LIPSa, and a division of RSTS into anterior (RSTSa) and posterior (RSTSp). Multidimensional scaling within these regions revealed the clearest distinction between high and low predacity in the RSTSa. In a complementary set of analyses, we mapped the brain for classification based on taxonomic class controlling for predacity. These analyses largely replicated our previous findings showing a separation of mammals, reptiles, and bugs along a lateral-to-medial animacy continuum that is most evident throughout ventral temporal and lateral occipital cortices. We further compared neural similarities across various brain regions with model similarity matrices for predacity, taxonomy, and early vision. The similarity analysis between regions and models show that RSTSa was most similar to the predacity model, followed by RSTSp, and LIPSa. The early visual regions identified in both the predacity and taxonomy analyses were closest to the early vision model, and the lateral occipitotemporal regions were most similar to the taxonomy model. In summary, these new findings suggest a distinct network spanning RSTS and LIPSa for representing the predacity of animals.

Poster 2

Do experienced meditators differ from non-meditators in emotion identification, competitive reactions, or compassionate responses

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Competition may reduce compassion via increasing testosterone and cortisol and impairing emotion identification. Conversely, meditation may increase compassion by stabilizing hormonal responses and enhancing emotion identification. This study compared meditators to non-meditators for emotion identification, hormone levels and changes to each following competition. Willingness to extend compassion despite hormonal fluctuations was also assessed. For baseline measurements, expert, novice and non-meditators completed a mindfulness survey, the Reading the Mind in the Eyes Task (RMET) of emotion identification and provided saliva. To increase testosterone and cortisol, participants won a competition against a confederate. Next, everyone began an arduous task; however, participants were later excused while confederates floundered. To assess post-competition changes in hormones and emotion identification, participants provided saliva and completed the RMET. To gauge compassion, participants were offered the opportunity to leave or anonymously assist the confederate. Pre-competition, expert meditators were the most mindful but no better at the RMET than non-meditators. Hormones did not differ among groups, but differed by gender. Post-competition, testosterone remained unchanged while cortisol increased; however, RMET scores did not change. Almost all participants assisted the confederate. In this study, meditation experience was not significantly correlated with enhanced emotion identification, hormonal fluctuations, or willingness to show compassion.

Poster 3

The effect of early life adversity on brain development in children and adolescents

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Experiencing early life adversity – neglect, abandonment, verbal, physical, and/or sexual abuse – increases a child's risk for psychiatric illnesses such as substance use disorder, anxiety and depression, as well as abnormalities in electrical brain activity (MacFarlane et al., 2005). Previous work using resting state electroencephalography (EEG) has shown that children exposed to adverse environments exhibit decreased cortical alpha, beta, theta, and delta power compared to children that were not exposed to adverse environments early in life (MacFarlane et al., 2005, Marshall et al., 2008). Further, children exposed to adversity also exhibited decreased cortical reactivity on auditory oddball and emotional Go/NoGo event-related potential (ERP) tasks (Pollak at al., 2001). The purpose of the present study is to examine the relationship between the degree of early life adversity (i.e. number of adverse events a child has experienced) and cortical EEG abnormalities. To address this relationship we use a quantitative measure of adversity, the Yale-Vermont Adversity in Childhood Scale (Y-VACS), and EEG recordings of resting-state, auditory oddball, and emotional Go/NoGo EEG on children ages 9-14. Preliminary findings indicate that the amount of trauma a child has experienced is negatively correlated with (A) P3 ERP amplitude on emotional Go/NoGo tasks, and (B) slow-wave relative power during eyes open resting state. Future research in this project will include analysis of EEG abnormalities with levels of attention problems, anxiety and depression symptoms, risk for substance use disorders and other behavioral characteristics. Also, future studies will examine the EEG connectivity differences associated with early life adversity and the relationship between EEG connectivity and MRI functional connectivity.

Poster 4

Role of prelimbic and infralimbic regions of medial prefrontal cortex in extinction and renewal of extinguished appetitive instrumental responding.

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The prelimbic (PL) and infralimbic (IL) sub-regions of medial prefrontal cortex (mPFC) have been shown to play critical, and possibly opposing roles in instrumental extinction and renewal in tasks using drug reinforcers (e.g., Bossert et al., 2011; Peters, LaLumiere, & Kalivas, 2008; Willcocks & McNally, 2013). However, the precise roles of these regions in extinction and renewal of instrumental behavior for non-drug reinforcers are not certain. Renewal of an extinguished instrumental behavior occurs when an animal is exposed to a context that is different from the extinction context (Bouton & Bolles, 1979). Renewal is a commonly used model of relapse in addiction, and as such understanding the underlying mechanisms is of particular interest and importance. Rats underwent acquisition of lever pressing for sucrose pellets in context A, followed by extinction (during which a lever press resulted in no reinforcer) in context B. Just prior to test sessions in context A and context B, rats received an infusion of $GABA_{A/B}$ agonist muscimol/baclofen (M/B), which temporarily inactivates neural tissue, or saline (control) in either PL or IL mPFC. Testing consisted of exposure to both contexts A and B with no reinforcers available. PL infusions of M/B prior to test decreased responding significantly in context A (the acquisition context), though both M/B and saline infused rats responded significantly more in context A than in context B (the extinction context), indicating renewal of responding. Infusions of M/B into the IL resulted in decreased responding in context A and increased responding in context B relative to controls. To confirm both cannula placement and the extent of infusion spread, fluorophore conjugated muscimol was infused in a subset of rats after testing. Visual analysis indicates that the infusions were confined to the PL or IL. These data suggest that the PL and IL are differentially involved in extinction and renewal of instrumental responding for a non-drug reinforcer.

Poster 5

Determining Shared Working Memory Systems for Rhyhtmic Incongruities in Music and Language Using Functional Near-Infrared Spectroscopy

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Rhythmic organization of auditory information is used differently in the retention of music and spoken language. However, similar areas of the prefrontal cortex (PFC) have been implicated in the retention of unusual rhythmic patterns. This study investigated the degree of PFC activation using functional near-infrared spectroscopy (fNIRS) during three rhythmic pattern manipulation working memory tasks. In addition the normalized pairJ wise variability index (NPVI) was tested as a measure of rhythmic accuracy. Of the 6 participants considered, 3 demonstrated greater activation of the right PFC in response to the Rhythmic Motor task, a manipulation of musical rhythms. Similar activation was observed for the Stress Speech task, which altered stress patterns in natural speech. No changes in activation were observed in the Rhythmic Speech task, which paired speech with metric patterns. The NPVI values did not reflect task performance. Refinement is needed to determine if the current procedure accurately measures rhythmic working memory.

Poster 6

Reducing Relapse by Manipulating the Temporal Distribution of Reinforcers in an Animal Model of Contingency Management Treatment

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Resurgence is sometimes considered a model of the relapse that occurs when reinforcement is discontinued after the conclusion of a contingency management treatment. Resurgence experiments conducted in the animal laboratory involve three phases. During Phase 1, an operant response such as a lever press (R1) is reinforced with a food pellet; in Phase 2, it is then extinguished (allowed to occur without its reinforcer) while a new behavior (R2) is introduced and provides the animal an alternative source of reinforcement. Then, in a third and final phase (the resurgence test), R2 is also placed on extinction, and responding on the original response (i.e., R1) resurges despite remaining on extinction. Several theories have attempted to explain the resurgence effect. To distinguish between them, two experiments with rat subjects examined how the temporal distribution of reinforcers delivered over the series of Phase 2 sessions affect resurgence when they are removed during the resurgence test. In general, any distribution of reinforcers that gave the subjects an opportunity to learn to inhibit performance of R1 when alternative reinforcers were sparse reduced the resurgence effect. The results are most consistent with the view that resurgence is a special type of "renewal effect" in which inhibited R1 responding recovers when the context provided by reinforcer presentations is removed. Resurgence, and by extension relapse that can occur after contingency management treatments, can be weakened when R1 is extinguished in a context that generalizes better to the context that prevails during resurgence (and relapse) testing.

Poster 7

An associative analysis of instrumental behavior chains

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Behavior often consists of sequences or "chains" of behaviors that include a procurement behavior followed by consumption. Behaviors that negatively impact health (e.g., drug abuse, smoking, or overeating) are typically part of such a chain. In any of them, performance of a procurement behavior in the presence of a procurement stimulus or situation (e.g., buying cigarettes or chips at a minimart) allows performance of a consumption response (e.g., smoking or eating) in the presence of its own stimulus (e.g., a pack of cigarettes, a bag of chips). Although the analysis of unhealthy behavior in terms of behavior chains has face validity, in contrast to simpler forms of instrumental conditioning, the associative structure underlying instrumental chains is poorly understood. We are interested in how behaviors situated in such chains can be inhibited or suppressed. Four experiments with rats were therefore conducted to begin to address the gap in our understanding. In each, rats were trained to perform a procurement response (e.g., lever press) in the presence of one stimulus to produce a second stimulus which signaled that a consumption response (e.g., chain pull) would yield a food-pellet reinforcer. In each experiment, rats then learned to inhibit one part of the chain (either the procurement or the consumption behavior). The results suggest that inhibition of the procurement behavior also suppressed the consumption behavior when consumption was tested in isolation. In addition, inhibition of the consumption behavior likewise suppressed procurement behavior when it was tested separately. The results provide evidence of a highly specific associative structure underlying behavior chains and suggest that treatments that seek to inhibit or suppress performance of one part of a chain might also serve to suppress others.

Poster 8

Melanocortin Receptor Expression After Early Life Seizures

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There is a well-described association between pediatric epilepsy and pervasive cognitive and behavioral deficits (Brunquell et al, 2002; Holmes et al, 2009), including a high incidence of autism spectrum disorders and attention-deficit hyperactivity disorder (Davies et al, 2003; Brooks-Kayal et al, 2010; Dunn et al, 2003). These psychiatric comorbidities are often more detrimental to quality of life for these patients and their families than the seizures themselves. Thus far treatment of these deficits has been primarily limited to treatment of the seizures themselves, with limited success, but targeting the deficits directly may prove more successful. We have previously shown that the hormone ACTH ameliorates attention deficits in an early life seizures (ELS) rat model without affecting interictal spike activity (Hernan et al, 2014), but the mechanism of its action remains unclear. ACTH is a ligand of the G-protein coupled receptor family of MCRs, two of which (MC3R & MC4R) reside in the CNS; these are naturally of interest when searching for ACTH's mechanism of action. Our experiment aims to confirm and measure expression of MC3R & MC4R in the PFC and ventral hippocampus of control and ELS rats using immunohistochemical staining and cell counting. Our hypothesis is that MCRs are highly expressed in these two brain regions and that expression of MCRs in both PFC and hippocampus will be altered after ELS. These results will provide further evidence for involvement of the melanocortin system in cognitive deficits associated with pediatric epilepsy and may support a role for targeting this system in the treatment of these deficits.

Poster 9

Environmental Enrichment Improves Hippocampal Networks in Animals with Malformations of Cortical Development

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Learning and memory deficits have frequently been observed in children with malformations of cortical development (MCD), a phenomenon often associated with childhood epilepsies. In these patients, cognitive deficits may result from a complex interaction of etiology (MCD), seizures, and the unintended effects of antiepileptic drugs used to treat these seizures. However, evidence increasingly suggests that seizure-focused pharmacological treatments do little to alleviate the behavioral and cognitive aberrations seen in epilepsy. Previous research in our lab has used methylazoxymethanol acetate (MAM), a DNA-methylating agent, to induce signature features of MCD in rodents; reduced cortical lamination, disruption of neuronal migration, and resistance to anticonvulsants. These animals also have significant deficits in spatial memory. We found that deficits in spatial navigation in rats exposed to MAM at embryonic day 17 were improved by environmental and social enrichment. Additionally, environmental enrichment normalized neuronal oscillatory activity and increased single unit activity in the hippocampus, an area traditionally associated with both spatial navigation and episodic types of memory. These findings point to demonstrable cellular and network-level phenomena in animals with MCD and hint at targets for potential treatment in patients with epilepsy. Preliminary data indicate that other aspects of cognition may also be affected in MAM animals. Future studies will examine the role of environmental enrichment in ameliorating these deficits as well.

Poster 10

CA1 Nav1.1 knockdown alters spatial information coding in the hippocampus

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Voltage-gated sodium channel Nav1.1 (coded by SCN1A gene) is important for maintaining fast neuronal firing throughout the CNS. In the hippocampus, fast firing is the hallmark of GABAergic interneurons. In CA1, interneurons regulate pyramidal cell (including place cells) activity and maintain hippocampal theta rhythm. The aim of this study is to investigate the consequences of Nav1.1 loss on hippocampal function. We hypothesize that Nav1.1 loss of function will lead to a drastic diminution of fast firing activity that will affect place cell properties and hippocampal oscillations. This could be the neural mechanism underlying spatial representation deficit and cognitive impairment observed in different neurological disorders (Dravet Syndrome, Autism, Alzheimer disease) all of which are associated with SCN1A mutations. Using a ShRNA interference approach, we induced a local Nav1.1 down regulation in the CA1 area in adult rats. In-vitro experiments show that NaV1.1 deficits in CA1 affect the firing of interneurons. In-vivo single-unit recordings in freely moving rats reveal that firing properties of both interneurons and pyramidal cells have been affected. Overall our data show that Nav1.1 knockdown in the CA1 alters both the rate and the temporal coding of the place cells. All together these data show that Nav1.1 deficit in CA1 induces strong network alterations underlying spatial information coding and memory.

Poster 11

Head direction cell activity in the dorsal striatum and medial precentral cortex requires intact anterodorsal thalamic nuclei

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Animals must maintain a sense of direction to effectively navigate within their environment. At the neural level, direction is represented by the activity of head direction (HD) cells. These neurons fire as a function of the animal's allocentric directional heading, operating much like a compass. While most rodent HD cells are located within the limbic system structures that form the HD circuit, small numbers are found elsewhere in the brain, including the dorsal striatum (DS) and medial precentral cortex (PrCM). Is the HD signal in these regions derived from limbic HD circuit output or is it generated independently? To examine this issue we recorded single unit activity in the DS and PrCM of freely moving rats and compared HD cell activity observed in control animals to that observed in animals with neurotoxic lesions of the anterodorsal thalamic nuclei (ADN), a manipulation known to disrupt the HD circuit. Large ADN lesions (> 85%) completely abolished the HD signal in both the DS and PrCM. Animals with smaller lesions exhibited degraded HD cell activity in the DS; these HD cells fired over a significantly wider directional range compared to HD cells from control animals. No HD signal was identified in the PrCM of any lesioned animal. Interestingly, units modulated by the animal's angular head velocity (AHV) were found in the DS and PrCM; unlike HD cells, the activity of these units was unaffected by ADN lesions. We conclude that the HD signal is first generated by the limbic HD circuit and then projected to the DS and PrCM from the ADN, the medial entorhinal cortex and/or the retrosplenial cortex. The AHV signal we observed in the DS and PrCM must either arise from a separate pathway, possibly a subcortical pathway involving the habenula, or be generated internally.

Poster 12

Evoked cortical potentials associate with center of mass displacement in response to an induced loss of standing balance

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Background: Induced loss of standing balance evokes cortical potentials that can be recorded by electroencephalography (EEG). The functional relevance of these cortical potentials to balance recovery remains unclear because they have never been correlated to the balance recovery response.
Objective: We sought to determine if the evoked N1 cortical potential relates to the magnitude of induced body displacement.
Methods: Twelve healthy subjects (eight females and four males, ages 22-50 yr, mean = 35 yr) responded to an induced loss of balance while standing on a platform that randomly rotated either "toes up" or "toes down" to five degrees with peak velocity of 23 degrees per second and a duration of 490 ms. Passive-marker motion capture was used to estimate center-of-mass (CoM) displacement from the initial point of equilibrium. Scalp EEG was used to derive the peak negative amplitude of the EEG voltage signal following the perturbation (the N1 potential). Pearson's correlation coefficients were used to correlate CoM displacements with N1 potential amplitudes.
Results: The N1 potential significantly correlated with CoM displacement in the "toes down" condition only at the Pz electrode ($r = -0.583$, $p = 0.047$), with near-significant correlations at the CPz ($r = -0.536$, $p = 0.072$) and Cz electrodes ($r = -0.533$, $p = 0.074$).
Conclusions: The N1 potential appears related to the extent of induced body displacement, which is most readily detected over midline sensory cortex when responding to a forward loss of balance.

Poster 13

KCNS1 as a Biomarker for Pain Perception in Patients with Chronic Musculoskeletal Pain

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Objectives: The primary goal of this research is to determine whether an allele within the gene encoding for the potassium channel alpha subunit KCNS1 is associated with increased pain perception in patients with chronic musculoskeletal pain. Previous research demonstrated that the potassium channel encoded by this gene plays a role in neuronal excitability and that expression in sensory neurons is substantially down-regulated in patients with neuropathic pain with Val substitution. Specifically those with homogenous Val/Val missense single nucleotide polymorphism (SNP) at the rs734784 KCNS1 allele experience increased pain sensitivity. Given this, we aim to: (1) determine whether the distribution of KCNS1 genotypes differs in our sample of chronic musculoskeletal pain patients compared to the general population, (2) identify physical and psychological characteristics of patients with musculoskeletal pain by KCNS1 genotype, and (3) to investigate whether clinical response to Cognitive Behavioral Therapy (CBT) for coping with chronic pain differs based on KCNS1 genotype. We hypothesize that patients with the Val/Val SNP will report greater pain sensitivity and show greater improvement post-CBT.

Methods: 201 adult male and female patients with chronic musculoskeletal pain were recruited to participate in this study. All participants underwent clinical evaluations and saliva samples were collected for DNA analysis. Clinical evaluations included a battery of behavioral inventories. After consenting patients were randomly assigned to either eleven weeks of group CBT or an attention control group. Ongoing data analysis includes creation of statistical models to determine the interaction of characteristics impacting pain experience.

Results: Preliminary analysis of these data revealed that the frequency of Val/Val substitution in our sample closely represents that of the general population (20.4% vs. 20.5%). In addition, at baseline, patients homogenous for the Val/Val substitution had lower pain levels ($p=.02$), higher SF-36 mental component scores ($p=.04$) and reported reduced catastrophizing ($p=.006$) compared to patients with no Val substitution.

Conclusion: These findings demonstrate that genetic variations in the KCNS1 allele correlate with pain perception. Contrary to our hypothesis, musculoskeletal pain patients with homogenous Val/Val mutations report lower average pain than those with no Val substitution, suggesting KCNS1 plays a different role in pain perception than in neuropathic pain. Work is underway to assess whether differences in genotype can be correlated with treatment outcomes.

Poster 14

Evaluation of White Matter Architecture Across Different Diffusion-Weighted Imaging Acquisition and Voxel-wise Reconstruction Methods

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Diffusion weighted imaging (DWI) is a method for visualizing white matter architecture *in vivo*. Diffusion tensor imaging (DTI) is the most common method due to its simplicity and parsimonious analysis, however, it may sometimes oversimplify tissue microstructure. Recently, advances in acquisition and reconstruction methods, such as diffusion spectrum imaging (DSI) and high angular resolution diffusion imaging (HARDI), allow for more extensive sampling of q-space and utilize model-free reconstructions. These techniques aim to more accurately capture intravoxel complexities, but may be more susceptible to noise. We evaluated DTI, HARDI, and DSI acquisitions under a single tensor model (DTI) and two model-free methods (DSI, generalized q-space imaging: GQI) in human and phantom data to compare estimations of fiber length and fiber crossing. Our results demonstrated that the DTI reconstruction provided more accurate estimates of fiber length in a region of the phantom that lacks crossing fibers. In human subjects, different reconstructions yielded significantly different estimates of fiber length ($F(4, 65)$=64.574, p<0.001), with model-free methods producing more accurate measurements. Results of our analyses of fiber crossing in the phantom also supported greater accuracy for the model-free approaches. A complementary qualitative analysis conducted on the human participants in the centrum semiovale, a neural junction that is known to have complex fiber crossings, supported the phantom findings. These findings suggest that the tensor model performs well for simple fiber orientations, whereas model-free reconstruction methods may better capture white matter connectivity in pathways with more complex geometric structure.

Poster 15

Variation in ethanol sensitivity in the Diversity Outbred mouse population: implications for genome-wide association mapping

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Alcohol use disorders (AUDs) are extremely deleterious conditions to both mental and physical health. Additionally, they are costly and problematically prevalent, affecting approximately 7.4% of the population of the United States. A strong predictor for future AUD onset is initial sensitivity to the intoxicating effects of alcohol. Alcohol sensitivity is a complex trait, arising from both environmental and genetic factors. To determine specific genetic contributors to ethanol sensitivity, we conducted three measures of intoxication in the JAX Diversity Outbred (DO) mouse population, a recently developed model population for use in high-resolution association mapping. Two hundred and twenty-four subjects were tested for ethanol-induced ataxia, ethanol-induced hypothermia, and ethanol-induced loss of righting reflex, each test assaying for a particular aspect of ethanol sensitivity. Due to the extensive genetic diversity present in the DO population, we observed increased variation in behavior as compared to inbred strains, suggesting that specific genetic variation may be correlated with varying levels of sensitivity to ethanol. With this behavioral data, it will be possible to correlate aspects of ethanol sensitivity with particular genetic loci of interest using the MegaMuga recombinant mouse genotyping array, which will allow genotyping at 77,000 single-nucleotide polymorphisms. This high-resolution genome-wide association will permit us to precisely pinpoint small sets of genes instead of the broader chromosomal regions traditionally found through association studies in mice. With further exploration, employing techniques such as RNAseq, we will correlate DNA sequence, behavior, and gene expression, eventually identifying a few genes that will enable us to better target mechanisms and risk factors that lead to AUD onset.

Poster 16

Identifying genes that contribute to conditioned fear in mice: a novel, forward genetic approach that may have implications with PTSD in humans

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Although posttraumatic stress disorder (PTSD) has been consistently shown to be genetic in nature, it has been difficult to reliably pinpoint particular genes associated with the condition. PTSD can be modeled in a conditioned fear (CF) paradigm in a simpler organism, the mouse. We have taken advantage of the newly created JAX Diversity Outbred Mouse Population (DO) to fine-map quantitative trait loci (QTLs) affecting naturally occurring variability in CF. The DO is a powerful population for fine mapping QTLs because they possess large amounts of genetic recombination similar to that observed in human populations. These recombinations break down linkage disequilibrium (LD) between the QTL and surrounding markers, allowing for identification of the causal variants that influence quantitative traits. Through a genome-wide association study (GWAS), we hope to identify single-nucleotide polymorphisms (SNPs) to locate alleles highly correlated with CF. We will genotype mice at ~78K SNPs and collect tissue for RNA-seq, but we must first show that the population shows a distribution for CF phenotypes. To date, we have tested 220 males for CF, and have observed a range of freezing behavior. On training day one, freezing ranges between 0.0% and 51.9% (Mean = 17.9%, SD = 12.3%), freezing to cue on day two ranges between 0.0% and 83.9% (Mean = 32.2%, SD = 17.1%), and freezing to context on day three ranges between 0.0% and 57.5% (Mean = 16.7%, SD = 13.4%). Thus, the combination of LD breakdown and varied CF responses makes the DO a promising mapping population for GWAS.

Poster 17

PACAP signaling enhances BNST neuronal excitability and increases circulating corticosterone

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Pituitary adenylate cyclase- activating polypeptide (PACAP) and its cognate PAC1 receptor have been associated with several mental health disorders that are related to stressor exposure and/or the dysregulation of the hypothalamic- pituitary- adrenal (HPA) axis, including anxiety and depression. In rats, exposure to repeated variate stress increased PACAP and PAC1 receptor expression in the bed nucleus of the stria terminalis (BNST). Moreover, intra-BNST PACAP infusion caused increased anxiety-like behavior and anorexic behavior, and BNST PACAP receptor antagonism attenuated many of the consequences of stressor exposure. Therefore, stress related consequences may be due to increased activation of BNST cells by PACAP. In the present set of studies, we utilized whole-cell patch-clamp electrophysiological techniques in BNST slices and observed enhanced excitability in a subset of BNST neurons that is consistent with an enhancement of the hyperpolarization activated cation current Ih. Furthermore, BNST PACAP infusion also resulted in increased plasma corticosterone levels to stress levels in both male and female rats, and this effect was not dependent on estradiol levels in females. These results support our work suggesting that BNST PACAP plays a key role in regulating stress responding and that dysregulation in BNST PACAP systems may play a critical role in some stress- related psychopathologies.

Poster 18

Intra-BNST PACAP Reinstatement to Cocaine Seeking in Rats

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Adequate treatment of cocaine addiction in humans is severely hindered by the tendency of users to relapse. For many cocaine-dependent individuals, physical life stressors can be important factors that contribute to increased use of the drug and chances of relapse. This paradigm can be modeled in rodents using a procedure in which stressor-exposure reinstates previously extinguished drug-seeking behavior. Here, we examined the role of pituitary adenylate cyclase activating peptide (PACAP) in the reinstatement of cocaine seeking in rats. We have previously shown that the activation of PACAP systems in the bed nucleus of the stria terminalis (BNST) mediates many of the consequences of stressor exposure, and BNST PACAP infusion can produce many behavioral and physiological stress responses. Hence, the current study was designed to determine whether BNST PACAP could reinstate extinguished cocaine seeking. Rats were allowed to self-administer cocaine (3mg/ml; 0.5mg/ig/infusion, i.v.) for 1 hr daily for 10 days and were then placed on an extinction schedule in which lever pressing no longer resulted in cocaine reward. Tests for reinstatement were administered after intra-BNST infusion of 1 μg PACAP 1-38 (0.5 μl per side) or equivolume vehicle. PACAP substantially reinstated lever pressing on the lever previously associated with cocaine delivery. These data suggest that BNST PACAP plays an important role in stress-induced reinstatement to drug seeking. Future experiments will utilize the same acquisition and extinction paradigms both to fully explore the neuropharmacology of BNST PACAP on stress-induced reinstatement and to determine whether PACAP systems represent a viable target for relapse prevention.

Poster 19

Parabrachial nucleus (PBn) PACAP projections to the lateral capsular division of the amygdala: modulatory roles in the sensory and behavioral aspects of pain

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The intricate relationships that associate pain, stress responses and emotional behavior are well established. Chronic pain can heighten the emotional and behavioral consequences of stress, as well as enhancing other pain experiences, which might be reflected in the comorbidity of chronic pain with a number of emotion-related pathologies including depression, anxiety abnormalities and disorders including posttraumatic stress disorder (PTSD). The central nucleus of the amygdala (CeA) represents a convergence of pathways for pain, stress and emotion. Among many amygdala neuropeptides, we have identified pituitary adenylate cyclase activating polypeptide (PACAP) immunoreactivity in fiber elements in the lateral capsular division of the CeA (CeLC). PACAP staining patterns colocalized in part with those for calcitonin gene related peptide (CGRP); anterograde fiber tracing and excitotoxic lesion studies demonstrated that the CeLC PACAP immunoreactivities represented sensory fiber projections from the lateral parabrachial nucleus (LPBn) along the spino-parabrachioamygdaloid tract. PACAP injections into the CeA increased anxiety-like behaviors accompanied by weight loss and decreased feeding, similar to previously described anxiogenic actions in the bed nucleus of the stria terminalis (BNST). In parallel, CeA PACAP signaling altered nociceptive responses as reflected by decreased response latency in thermal sensitivity tests, an effect that was replicated with the PACAP PAC1 receptor agonist maxadilan. The current observations suggest a role for CeA PACAP signaling in pain responses and emotional behaviors. Within the spino-parabrachioamygdaloid tract alterations of PACAP and other neuropeptide signaling may represent neuroplastic mechanisms that associate chronic pain with sensory hypersensitivity, heightened states of anxiety, and severe behavioral disorders.

Poster 20

Differential mechanisms of vasodilation of PACAP and CGRP in rat middle meningeal artery: Potential role in migraine headache

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Migraine is the most common incapacitating neurological disorder, characterized by an intense pulsating headache. The cellular mechanisms contributing to migraine headache are poorly understood, but a leading hypothesis is that prolonged dilation of cranial arteries, specifically the middle meningeal artery (MMA), is involved in the sensation of headache pain. The neuropeptides pituitary adenylate cyclase activating polypeptide (PACAP) and calcitonin gene-related peptide (CGRP) have been shown to potently dilate the MMA and induce migraine-like headaches. The mechanisms by which these peptides exert their effect on the MMA remain unclear. The goal of this study is to decipher the mechanisms of PACAP and CGRP-induced dilations using freshly isolated pressurized rat MMAs. At an intravascular pressure of 40 mmHg, MMAs developed myogenic tone (i.e. constricted) representing ~ 40 % decrease in diameter. Treatment of these arteries with PACAP (3 nM) or CGRP (1 nM) caused significant vasodilation (59 ± 5.8 % of maximum and 77 ± 4.01 % of maximum, respectively). PACAP-induced dilation was abolished in the presence of glibenclamide, an ATP-sensitive K+ (KATP) channel blocker. However, CGRP-induced dilation remained unaffected by treatment with glibenclamide, alone. Paxilline, a blocker of large-conductance Ca2+-activated K+ (BK) channels, or 4-aminopyridine, a blocker of voltage-gated K+ (KV) channels also did not affect CGRP-induced MMA dilation. Further, CGRP-induced dilations were not altered by a combination of L-nitroarginine (L-NNA) to inhibit nitric oxide synthesis, indomethacin to inhibit prostacyclin synthesis, apamin to block endothelial small-conductance Ca2+-activated K+ channels and TRAM-34 to block endothelial intermediate-conductance Ca2+-activated K+ channels. Interestingly, CGRP-induced dilations were blocked by raising extracellular K+ to 30 mM, implicating involvement of K+ channel activation in the dilatory response of this peptide. Further, CGRP-induced dilations were abolished by a combination of compounds that included glibenclamide, paxilline, L-NNA, indomethacin, apamin, TRAM-34 and thapsigargin, an inhibitor of the sarco-endoplasmic reticulum Ca2+-ATPase. In summary, although PACAP and CGRP have been reported to increase adenylyl cyclase activity, they act via distinct vasodilatory mechanisms in the MMA. PACAP induces vasodilation through KATP channel activation, while CGRP appears to utilize multiple cell signaling pathways. By understanding the distinct mechanisms involved in MMA dilation caused by PACAP and CGRP it may be possible to develop new combination therapies for migraine headache.

Poster 21

Measuring Changes in Surface Kv1.2 Expression in Cerebellar Cortex following Eyeblink Conditioning

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Eyeblink conditioning (EBC) is a well-studied form of classical conditioning supported by plasticity in the cerebellum. EBC involves trials in which a tone conditioned stimulus (CS) is followed by an eyelid stimulation unconditioned stimulus (US). The result of conditioning is an eyeblink conditioned response (CR), which occurs in response to the CS prior to the onset of the US. Both Purkinje cells (PCs) and neurons of the interpositus nucleus (IPN) receive CS and US inputs; in order for a CR to be exhibited, the tonic inhibition of the IPN neurons from PCs needs to be lifted. Our model proposes that the regulation of Kv1.2, an α-subunit of Kv1 voltage-gated potassium channels that is densely expressed on basket cell (BC) axon terminals and PC dendrites in the cerebellar cortex, is integrally important through a feed-forward inhibitory pathway involving parallel fibers, BCs and PCs. BCs are innervated by CS-carrying parallel fibers. It was previously shown that blocking Kv1.2 increases inhibitory currents in PCs. Additionally, we have shown that blocking Kv1.2 through intracerebellar infusions of tityustoxin (TSTX), a selective blocker of Kv1.2, dramatically enhanced acquisition of CRs. We also showed that intracerebellar infusions of secretin, a retrograde messenger released by PCs that reduces surface Kv1.2 at BC-PC synapses, facilitated conditioning. Finally, we showed that intracerebellar infusions of a secretin receptor antagonist impaired conditioning. These findings led us to hypothesize a reduction in the surface expression of Kv1.2 in BC axon terminals during EBC. In order to test this hypothesis, rats were assigned to one of three groups: Paired (100 CS-US paired trials on each day), Unpaired (100 CS-alone & 100 US-alone trials intermixed on each day), or No Stimulus (100 no stimuli trials on each day), and immediately after the last of three days of conditioning, a portion of the lobulus simplex region of cerebellar cortex was fixed, harvested, and sectioned. Sections were assigned to one of two methods for analysis of cerebellar Kv1.2 surface expression: biotinylation and western blot or multiphoton microscopy. Preliminary data show strong learning, in the form of eyeblinks to the tone CS, in the Paired group and very little responding in the Unpaired and No Stimulus groups. In the western blot data, we observed greater Kv1.2 surface expression in the Unpaired group compared to the Paired and No Stimulus groups, which did not differ. The biotinylation data conflates PC and BC Kv1.2, and these data are clarified by the microscopy data, where we observed lower Kv1.2 surface expression in the BC axon terminals in the Unpaired group compared to the other two groups. Since Kv1.2 is expressed in both BC axon terminals and PC dendrites, this pattern of results suggests that mere exposure to the stimuli used in EBC decreases surface Kv1.2 at BC axon terminals but increases surface Kv1.2 at PC dendrites and this latter increase is of greater magnitude. We hypothesize that once a predictive association between the tone and eyelid stimulation is learned, surface Kv1.2 returns to its homeostatic level.

Poster 22

Plasticity in ion channel expression (BKCa, SKCa) in micturition reflex pathways during postnatal rat development

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The storage and periodic elimination of urine exhibit marked changes during prenatal and postnatal development but the mechanisms underlying these changes are largely unknown. In the young fetus, prior to maturation of the nervous system, urine is presumably eliminated from the urinary bladder by non-neural mechanisms. As the central nervous system continues to mature during the postnatal period, reflex voiding is brought under voluntary control involving higher brain centers. Both large-conductance (BK) and small potassium-conductance (SK) channels are expressed in adult bladder and underlie the contractile and electrical activities of the urinary bladder. Three isoforms of the Ca2+-activated, voltage-independent SK channels have been identified: SK1, SK2 and SK3. In neurons, the SK channels also contribute to the action potential afterhyperpolarization and are essential to reduce action potential frequency and neuronal excitability. Inhibition of SK channels increases urinary bladder smooth muscle excitability, contractility and neuronal excitability. Developmental- and bladder dysfunction-induced plasticity of K+ channels and specifically, KCa channels, has been shown in autonomic neurons, lumbar motoneurons and urinary bladder. We are considering plasticity in ion channel expression (BKCa, SKCa) and/or distribution as an underlying mechanism regulating maturation of micturition reflexes and reemergence of immature voiding reflexes following injury. Using quantitative polymerase chain reaction and immunohistochemical approaches, we examined ion channel expression (BKCa, SKCa) in urothelium+suburothelium and lumbosacral DRG in postnatal rats. Expression of the urothelium transcripts is expressed relative to the reference gene, 18S. SK1, SK2 and SK3 and BKα and BKβ mRNA expression was observed in urothelium of all postnatal ages (P3, P5, P10, P12, P21, Adult) examined. SK1, SK3 and BKβ mRNA exhibited decreased expression with postnatal maturation (R2 = 0.996-0.998). BKα exhibited a biphasic mRNA expression with postnatal development. In contrast to mRNA expression in the urothelium, SK1-SK3 and BK mRNA expression increased in L6-S1 and L1-L2 DRG with postnatal maturation. BK mRNA expression exhibited a biphasic expression in L6-S1 and L1-L2 DRG with postnatal maturation. Immunoreactivity for SKCa isoforms was demonstrated in wholemounts of urothelium and in the suburothelial nerve plexus in all postnatal ages examined. BK1-IR was also demonstrated in urothelium wholemounts but staining of nerve fibers in the suburothelial plexus was not observed in all postnatal ages examined. Functional contributions of ion channel expression (BKCa, SKCa) to the maturation of voiding reflexes are being examined.

Poster 23

The contribution(s) of transforming growth factor-beta to bladder afferent nerve hyperexcitability with cyclophosphamide-induced cystitis

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Bladder Pain Syndrome (BPS)/Interstitial Cystitis (IC) is a major unresolved health concern in the United States with a considerable economic burden in lost productivity and health care. We hypothesize that the pleiotropic protein, transforming growth factor-beta (TGF-β), and its cognate receptors contribute to afferent nerve hyperexcitability that may facilitate bladder hyperreflexia in an experimental cystitis model of BPS/IC. To determine the role of TGF-β in bladder afferent nerve hyperexcitability we used an ex vivo bladder-pelvic nerve preparation in control and CYP-treated tissues. Intravesical instillation of recombinant TGF-β in control tissues significantly increased mean afferent nerve frequency (imp/sec) at various bladder distentions. Forty-eight hours following CYP-induced cystitis, mean afferent nerve frequency (imp/sec) significantly increased relative to control tissues. The intravesical instillation of a TGF-β type I receptor inhibitor significantly decreased the enhanced mean afferent nerve frequency (imp/sec) in CYP-treated tissues. Given its spatiotemporal regulation in the urinary bladder inflammatory response, these results suggest TGF-β may, in part, play a role in the development of lower urinary tract symptoms in BPS/IC by contributing to afferent nerve hyperexcitability and peripheral and central sensitization. Manipulation of this signaling pathway following bladder inflammation may provide a novel therapeutic opportunity to alleviate lower urinary tract symptoms.

Poster 24

Endothelin-1 potentiates Heparin-binding EGF-like growth factor-induced vasoconstriction in rat parenchymal arterioles.

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Background: Endothelin-1 (ET-1) is powerful spasmogen, and that increases in concentration in cerebrospinal fluid during vasospasm after subarachnoid hemorrhage have shown in previous reports. However, physiological concentration of ET-1 in cerebrospinal fluid is approximately 0.2 pM that cannot directly cause vasoconstriction, even at high concentrations (0.7 pM) during vasospasm neither. Meanwhile, the augmenting effect of low concentration of ET-1 to other vasoconstrictors has been reported. Our laboratory has recently reported that heparin binding epidermal growth factor-like growth factor (HB-EGF) mediates oxyhemoglobin-induced vasoconstriction. Here, our objective was to investigate the ability of low concentrations of ET-1 to potentiate HB-EGF-induced vasoconstriction in rat parenchymal arterioles.

Methods: In the current study, we examined the ability of low concentration ET-1 to potentiate HB-EGF-induced constriction on parenchymal arterioles that were isolated from rats and pressurized ex vivo.

Results: ET-1 caused vasoconstriction on dose dependent manner (EC50 ~80pM). 3pM ET-1 which is close to maximum concentration in cerebrospinal fluid during vasospasm after subarachnoid hemorrhage, did not cause constriction in rat parenchymal arterioles but potentiated HB-EGF-induced constriction (with / without 3pM ET-1 : 11.6%/2.2 %). The thromboxane A2 analogue (U46619) and 30 mM K^+ that caused strong constriction in rat parenchymal arterioles did not potentiated HB-EGF-induced constriction. Suppression of voltage-dependent potassium (K_V) channels with 4-aminopyridine and inhibition of endocytosis with dynasore markedly abolished the potentiation of ET-1 to HB-EGF-mediated constriction.

Conclusion: We provide evidence that low concentration of ET-1 augments the action of HB-EGF to constrict parenchymal arterioles via a novel mechanism involving K_V channel endocytosis. This pathway may be involved in enhanced constriction of the brain microcirculation after SAH.

Poster 25

In Vivo and *Ex Vivo* Dysfunction of Neurovascular Coupling in a Mouse Model of Subarachnoid Hemorrhage

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Neurovascular coupling (NVC) represents activity-dependent focal increases in cerebral blood flow (CBF) crucial for the enhanced delivery of nutrients to maintain brain function in regions of high metabolic demand. We have previously reported inversion of NVC, with neuronal activation causing vasoconstriction rather than vasodilation, in brain slices from subarachnoid hemorrhage (SAH) model rats (Koide et al, 2012). Here, we examined ex vivo NVC, in vivo functional hyperemia and sensory motor function using a mouse endovascular perforation SAH model. In brain slices, astroctyic endfoot Ca2+ and adjoining parenchymal arteriolar diameter were measured using two-photon and infrared-differential interference contrast microscopy. Neuronal activation caused increased endfoot Ca2+ that was followed by an inverted neurovascular response (i.e. vasoconstriction rather than vasodilation) in ~80% of brain slices from day 1 and day 2 SAH mice and ~ 30% of slices from SAH day 4 mice. In vivo functional hyperemia (whisker stimulation-induced CBF increases) measured by laser Doppler flowmetry in SAH day 1 mice was also significantly attenuated. Consistent with impaired NVC, SAH mice showed a decreased ability to perform sensory motor tasks. These data demonstrate dysfunction of neurovascular coupling occurs both ex vivo and in vivo following SAH, which may contribute to the development of neuronal deficits.

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Poster 26

Brain-Derived Neurotrophic Factor Overexpression in the Bed Nucleus of Stria Terminalis has no Effect on Blood Pressure Regulation

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Psychological stress is an important risk factor for cardiovascular diseases, and brain-derived neurotrophic factor (BDNF) signaling within the bed nucleus of stria terminalis (BNST) has been implicated in mediating stress- and anxiety-like behavioral responses. Furthermore, we have shown recently that BDNF signaling in the paraventricular nucleus of the hypothalamus (PVN) is a regulator of cardiovascular function and sympathetic nervous system activity. However, it is unknown whether BDNF in the BNST is involved in neural regulation of cardiovascular function during baseline conditions or during stress. Therefore, the objective of this study was to determine if viral vector-mediated overexpression of BDNF within the BNST produces changes in resting blood pressure and alters hypertensive responses to acute stress. Male 8-week-old Sprague-Dawley rats were equipped with radiotelemetry transmitters for measurement of blood pressure. After one week of baseline blood pressure recording, rats received bilateral BNST injections of adeno-associated viral vectors (AAV2) expressing BDNF (n = 8) or green fluorescent protein (GFP; n = 7). Blood pressure was monitored for an additional 5-week period. During weeks 3 to 5, animals were subjected to two types of acute stress (restraint stress – 60 min; water stress – 1cm 25ºC water for 15 min) and open field and elevated plus maze behavioral tests to assess cardiovascular stress responses and anxiety-like behavior. Resting mean arterial pressure (MAP) did not change from pre-injection baseline values in either group, and daytime mean arterial pressure (MAP) averaged for week 2 to 4 after injections was 97 ± 1 mmHg in the GFP and 96 ± 2 mmHg in the BDNF group. Acute stress induced marked increases in MAP in both groups; however BDNF failed to have a significant effect on these responses. During restraint stress, peak MAP increases were 46 ± 2 mmHg in GFP vs. 39 ± 4 mmHg in BDNF rats, and average MAP increase during stress was 31 ± 1 mmHg in GFP vs. 25 ± 3 mmHg in BDNF rats. Similarly, there were no differences in peak MAP changes (40 ± 2 mmHg GFP vs. 36 ± 3 mmHg BDNF) or average MAP increase (34 ± 2 mmHg GFP vs. 28 ± 4 mmHg BDNF) during water stress. Behavioral tests indicated that there was no difference in the center/periphery ratio between GFP (0.05 ± 0.01) and BDNF (0.05 ± 0.01) groups during open field testing, nor was there a significant difference in the ratio of time spent in open/total arms of the elevated plus maze (0.38 ± 0.04 GFP vs. 0.29 ± 0.05 BDNF). While these data indicate that vector-mediated overexpression of BDNF within the BNST fails to affect resting blood pressure and stress-induced pressor responses, further studies are needed to examine the importance of stress-related rises in endogenous BDNF in the mediation of both cardiovascular responses and anxiety-like behavior elicited by stress.

Poster 27

Rho Kinase Regulates Myogenic Depolarization of Cerebral Parenchymal Arterioles

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While the mechanisms of tone regulation in cerebral pial arteries are well-described, much less is known about the vasomotor control of brain parenchymal arterioles (PAs). This is significant given the unique roles of PAs in the cerebral microcirculation, including control of local blood flow and perfusion pressure, and involvement in neurovascular coupling. Further, PAs are considerably more sensitive than pial arteries to increase in intravascular pressure, which causes smooth muscle depolarization and vasoconstriction (myogenic tone). A recently published study from our laboratory indicates that TRPM4 channels couple P2Y purinergic receptor mechanoactivation and myogenic tone of PAs. Based on recent observations that inhibiting protein kinase C has limited effects on PA myogenic constriction, the objective of the present study was to determine the roles of Rho-associated protein kinase signaling in TRPM4-mediated myogenic tone of PAs. Here we report that the Rho kinase inhibitor H1152 robustly inhibited pressure- and P2Y agonist-induced constriction of PAs. In contrast to the typical function of Rho kinase to alter Ca2+-sensitivity, we found that H1152 inhibited pressure-induced intracellular Ca2+ increases, and reduced UTPγS (P2Y4 receptor)- and UDP (P2Y6 receptor)-initiated Ca2+ entry in PA smooth muscle by 61% and 50%, respectively, suggesting that Rho kinase is centrally involved in myogenic depolarization and Ca2+ influx in PA smooth muscle. H1152 also reduced basal TRPM4 activity by 61%, and UTPγS- and UDP-activated TRPM4 currents by 75% and 73%, respectively. These results illustrate a novel role for Rho kinase in regulation of TRPM4-mediated depolarization, Ca2+ influx and myogenic tone in the cerebral microcirculation.

Poster 28

Umami taste potentiation in mice

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Taste is an important evolutionary adaptation that has allowed humans to select specific nutrients and avoid toxic ones (Eschle, et al., 2009). The five basic tastes that activate the gustatory part of the human and rodent nervous system are sweet, sour, bitter, salty and umami. The prototypical substance for umami taste is L-monosodium glutamate (MSG), but compounds such as inosine 5'-monophosphate (IMP) and other monophosphates have been shown to increase the intensity of umami taste perception through a synergistic mechanism. The resulting intensity is greater than the sum of the intensities of the L-amino acid and IMP (Kusuhara, et. al 2007; Wifall, et. al 2007; Yamaguchi, 1967; Yamamato et al., 1991; Yoshii, et. al 1986). Besides MSG, other L-amino acids also show synergism when mixed with IMP. We have previously shown that mice conditioned to avoid L-MSG will generalize that aversion to the amino acids L-arginine and L-serine (Viscido, 2014). This study expanded upon that research by using a conditioned taste aversion (CTA) paradigm in which a mixture of L-MSG mixed with IMP (CS) is paired with LiCl (a US that induces gastric malaise) to form a CTA in mice. The lick rates of these mice were then tested to see if this CTA generalized to L-serine mixed with and without IMP. The results suggest that mice conditioned to avoid an MSG solution mixed with IMP show a significant generalization of aversion to both L-serine mixed with and without IMP. Generalization was greater when IMP was added. These results suggest that IMP functions as a significant potentiator of umami taste leading to greater stimulus generalization. Future experiments will expand this research by conditioning mice to avoid L-serine and testing them with L-MSG.

Poster 29

The synergistic effects of minerals and lactic acid contributing to the taste of dried-bonito *dashi*

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Dried-bonito *dashi*, a Japanese fish stock, is an important component of Japanese cuisine and is a preferred flavor of humans and rodents. It is made of a complex mixture of amino acid, proteins, organic acids and minerals and has been shown to elicit all 5 of the basic tastes. The purpose of this study was to use condition taste aversion (CTA) methods to determine if an aversion to *dashi* generalizes to one or more of 4 salts (NaCl, KCl, $CaCl_2$, $MgCl_2$), and if lactic acid (a large component of *dashi*) alters the taste and resulting generalized aversion in C57Bl/6J mice with compromised olfactory systems. Conditioning and generalization testing were done with 25% solution of dashi (conditioned stimulus) presented in a Davis Rig (MS160). Generalization was measured by counting licks when mice were presented with NaCl (100 & 300 mM), KCl (100 & 300 mM), $CaCl_2$ (15 & 30 mM), and $MgCl_2$ (20 & 40 mM), with or without 0.9% lactic acid added. We found that all 4 salts showed mild naturally aversive qualities at their highest concentration. Additionally, *dashi* CTA generalized more to the divalent salts than the monovalent salts. Interestingly, lactic acid had little effect on CTA generalization to the monovalent salts whereas it decreased generalization to divalent salts. The CTA did not generalize to lactic acid alone. These results suggest that all 4 salts may contribute to the taste of *dashi* and that interactions between lactic acid and divalent salts alter the tastes elicited by these salts. Further studies will examine how the interactions between lactic acid, amino acids, inositol, and these salts may contribute to *dashi* taste.

Poster 30

The prototoxin LYPD6B modulates heteromeric alpha3 beta4 containing nicotinic acetylcholine receptors but not alpha7 homomers.

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Nicotinic acetylcholine receptors (nAChRs) are involved in a variety of processes such as neural differentiation, survival, attention, and memory. Proteins from the Ly-6/uPAR family also known as prototoxins, have been identified as accessory modulators of signaling through nAChRs; however, the specificity of prototoxins and their functions have yet to be thoroughly explored. We previously discovered that the prototoxins LYPD6B and PSCA are expressed in the avian ciliary ganglion. Since PSCA limits ACh induced activation of α7, we hypothesized that LYPD6B would modulate α3* containing nAChR heteromers that include α3, β4 and α5 subunits. To test this hypothesis, we determined whether the co-expression of LYPD6B with varying α3, β4 and α5 containing nAChR concatemers in Xenopus oocytes causes differences in ACh sensitivity (EC50), alters the maximum current induced by ACh (Imax) and/or changes the rate of desensitization (τ) caused by ACh. LYPD6B specifically enhances the EC50 and reduces the Imax elicited by ACh of α3β4 concatemers containing three α3 subunits. If one of the α3 subunits is replaced by a α5 subunit, there is no shift in the EC50 or τ caused by ACh but there is a decrease in the Imax. In contrast to α3* nAChRs, LYPD6B has no effect on α7 containing homomers. Thus, the effects of LYPD6B are limited to select stoichiometries of a3 containing nAChRs. These results suggest that the modulatory effects of prototoxins on nAChRs are complex and highly specific.

Poster 31

Cerebellar mGluR1 Modulates Cerebellar-Dependent Learning

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Eyeblink conditioning is a type of Pavlovian conditioning in which the unconditioned stimulus (US), conditioned stimulus (CS), and conditioned response (CR) pathways are well characterized. Across training sessions, rats receive many trials of conditioned stimulus and unconditioned stimulus pairings. In these trials, a tone (CS) predicts a periorbital shock (US) and rats learn to blink to the tone (CS) in anticipation of shock (US); this learned, anticipatory eye blink is the CR. Input from the CS and US are relayed separately through the brainstem and onto both Purkinje cells in cerebellar cortex and to one of the deep cerebellar nuclei, the interpositus nucleus. Plasticity at parallel fiber-to-Purkinje cell synapses in cerebellar cortex and mossy fiber-to-interpositus nucleus synapses underlies the CS-US association and expression of the eyeblink CR. In order for learning to occur, tonic inhibition of the interpositus nucleus by Purkinje cells must be lifted. Basket cells are inhibitory interneurons in the cerebellar cortex that strongly modulate Purkinje cell excitability. Kv1.2 is a potassium channel subunit that is expressed in basket cell axon terminals and Purkinje cell dendrites. Previous research in our lab has demonstrated that internalization of Kv1.2 coincides with an enhancement in eyeblink conditioning. Our working model of the role of Kv1.2 in eyeblink conditioning is that certain neuromodulators such as secretin can induce endocytosis of Kv1.2 channels at basket cell-Purkinje cell synapses. This causes a reduction in Purkinje cell excitability and disinhibition of the tonically inhibited interpositus nucleus, resulting in expression of a conditioned response (Williams et al., 2012). Recent research in our labs has shown that agonism of Group I metabotropic glutamate receptors (mGluR1/mGluR5), like agonism of secretin receptors, reduces surface expression of Kv1.2 in cerebellar cortex. Because suppression of Kv1.2 enhances EBC, we hypothesized that mGluR1 agonism would enhance eyeblink conditioning. To test this we infused the mGluR1/mGluR5 agonist DHPG into the lobulus simplex region of the cerebellar cortex prior to training sessions 1 and 2. Rats received 6 training sessions across 6 days with 80 paired trials, intermixed with 10 CS-alone and 10-US alone trials, per session. Preliminary results support our hypothesis. We are currently examining the effects of mGluR1 antagonism on eyeblink conditioning and preliminary data suggest an impairment of learning. Future research will examine whether mGluR1 activation in cerebellar cortex enhances eyeblink conditioning as a result of Kv1.2 regulation.

Poster 32

Regulation of microtubule dynamics by Tau

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Tau protein is implicated in numerous neurodegenerative diseases such as Alzheimer's disease (AD) and frontotemporal dementia. An intrinsically disordered protein, tau, performs a variety of functions in a neuron that are important for axonal transport. One of these functions is the regulation of microtubule dynamics. Despite numerous studies, the role of tau in the regulation of microtubule dynamics is still unclear. In the adult brain six isoforms of tau are expressed. In this study we aim to study the isoform specific regulation of microtubule dynamics by tau. We also seek to identify the mechanism by which tau regulates microtubule dynamics. To achieve our objectives, microtubules were polymerized, in vitro, in the presence of different isoforms of tau and TIRF microscopy was used to analyze microtubule dynamics at a single molecule level. Images were then analyzed using a custom code in Matlab software which used error function approximation to calculate the length of the microtubule and taper of the microtubule tips. In this way we are able to calculate dynamic parameters such as growth rate, shortening rate, catastrophe frequency and rescue frequency. Also, we can correlate microtubule tip taper to microtubule dynamics to see if tau regulates microtubule dynamics by altering the structure of the microtubule tip. We are in the process of analyzing our data.

Poster 33

The role of plexinA1 in visual system development of *Danio rerio*

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Plexin receptors, and their ligands, semaphorins, mediate axon growth in the visual system by facilitating actin cytoskeletal rearrangements and initiating growth cone collapse through intracellular signaling mechanisms. A proposed model for the development of the early eye involves the interaction of sema6A and plexinA2 on retinal precursor cells. However, this model is not fully explained by previous data; we propose that plexinA1a also plays a role. This research uses in situ hybridization, antisense morpholino gene knockdown, and site-directed mutagenesis with CRISPR technology to identify the expression and function of the plexinA1a receptor in early zebrafish eye development, which have yet to be characterized. We hypothesize that plexinA1a is expressed in the same tissues as plexinA2 and has a compensatory function.

Poster 34

Defining the role of Histidyl tRNA Synthetase in the Zebrafish Eye and Ear

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Aminoacyl tRNA synthetases are critical enzymes responsible for attaching specific amino acids to their appropriate tRNA molecules during protein synthesis. In humans, a point mutation in the gene for Histidine tRNA Synthetase (HARS) has been associated with Usher Syndrome Type 3b, a disease characterized by hearing and vision loss by early adulthood. This association suggests an important role for HARS in eye and ear development and maintenance. The use of antisense morpholinos to knockdown HARS expression in zebrafish results in a lack of retinal organization and loss of neuromasts – exterior sensory organs in the zebrafish that are comparable to the sensory patches in ears. These results support the hypothesis that HARS plays a particularly important role in the vertebrate eye and ear. We aim to define the role of HARS in these sensory systems using the zebrafish as a model.

Poster 35

Novel Tyrosine Phosphorylation Sites Fine Tune the Activity and Substrate Binding of the Src Family Kinase Fyn

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The protein Fyn is a member of the Src Family of non-receptor tyrosine Kinases (SFKs) that are important in many cellular processes including neuronal migration. Phosphorylation is an essential post-translational modification that has been previously shown to regulate kinase activity in SFKs. Many site-specific studies have identified tyrosine phosphorylation sites in SFKs that are both activating (e.g. Fyn Y420) as well as inhibitory (e.g. Fyn Y531) to SFK activity. These sites are located in the kinase domain and C-terminal regulatory domain of SFKs, respectively. More recently, we have utilized large-scale mass spectrometry based phosphoproteomic approaches and have identified novel phosphorylation sites in the SH2 (Src Homology 2) and kinase domain of Fyn that to date do not have known molecular functions. These sites are largely conserved in SFKs and across evolution. Using site-directed mutagenesis, we engineered constructs with partially phosphomimetic (Y to D) or non-phosphorylatable (Y to F) mutations for four of these sites, including Y185, Y213, Y214 (within the SH2 domain), and Y440 (within the kinase domain). Using in vitro and cellular approaches, we determined that SH2 domain phosphorylation at these sites increases Fyn's kinase activity while simultaneously reducing binding of the known Fyn SH2 binding partner, ESDN. Conversely, we determined that phosphorylation of Y440 in the kinase domain reduces overall Fyn kinase activity. These results suggest that these previously uncharacterized phosphorylation events fine-tune SFK activity.

Poster 36

A role for Fgf8a in neurovasculature signaling in the developing zebrafish retina

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Fibroblast growth factors (Fgfs) are critical in many aspects of embryonic development and other cellular functions including apoptosis, cell adhesion, and proliferation. We identified mRNA expression of Fgf8a in the presumptive retinal ganglion cells (RGCs) and its receptor FGFR1b in surrounding retinal vasculature of 2 day-old zebrafish. Acerebellar (Ace) mutants, lacking Fgf8a, show a significant reduction in eye diameter and also a reduction total cell number in the retina. In addition, Ace embryos have mispatterned retinal vasculature and a lack of blood flow through the eye suggesting a role in neurovascular signaling. It has previously been reported that zebrafish survive and develop normally for 7 days without blood flow as it receives nutrients by simple diffusion. To rule out hypoxia as a cause for the observed Ace mutant phenotype, we utilized the silent heart mutant fish line, which lacks cardiac troponin t resulting in embryos without blood flow, as heart contractility does not initiate. Cell counts from these fish have however, shown a decreased eye diameter and a loss in total cell numbers in the retina. Therefore, using immunohistochemistry, we looked to see if loss of cells was due to lack of proliferating cells using pHH3 or increased cell death using anti-active caspase 3 in both silent heart and Fgf8a mutant fish. We hypothesize that Fgf8a, from the RGCs, signals through Fgfr1b on the retinal vasculature to promote growth its growth and development. Subsequently, we suggest that the retinal vasculature responds by secreting an unknown factor to support the proliferation and maintenance of the RGCs. To further our understanding of this intricate developmental system we intend to look closer into the connection between the RGCs and the developing vasculature.

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University of Vermont COBRE Neuroscience Cell and Molecular Biology Core Facility

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The COBRE Neuroscience Cell and Molecular Biology (CMB) Core at the University of Vermont was established to serve the neuroscience community by providing the equipment and training to incorporate cell and molecular approaches into their research. The core personnel are the core director, Dr. Sheryl White, and two full time technicians: Thomm Buttolph and Edward Zelazny. The CMB core provides one of the widest ranges of molecular biology services available in academic facilities in the country, including DNA services (construct design, cloning, PCR, site-directed mutagenesis and library construction), RNA services (Quantitative PCR, RNA isolation, RT-PCR, Northerns, RNase protection and differential display analysis), protein services (SELDI-TOF mass spectrometry biomarker profiling, protein extraction, SDS-PAGE, 2D-PAGE, western blotting and gel shift assays), cell culture services (primary/cell line culturing, transfection, reporter assays, immunohistochemistry, frozen/paraffin sectioning and slide staining), as well as specialized microscopy techniques (laser capture microdissection, Neurolucida morphometrics, stereology and cell counting). The CMB core also offers training services to laboratory personnel or principal investigators wishing to learn molecular biology/cell culture technique. The equipment in the facility is available for researchers to use and includes specialized equipment such as two ABI 7500FAST systems for quantitative PCR, a SELDI-TOF mass spectrometer for proteomics and biomarker identification, a Zeiss-PALM laser microdissection system for isolation of single cells, and an Odyssey infrared imager system for Western blotting, gel shift assays and in-cell western analysis. Recent additions include a Qiagen 24 Pyrosequencer, Biotek SynergyH4 plate reader, a Countess automated cell counter, an MP Bio FASTPREP 24 cell and tissue homogenization system, Qiagen's QIAcube and a Qiagility liquid handling system. A full list of all the equipment and services available can be viewed online at UVM's Neuroscience Center of Biomedical Research Excellence webpage

Poster 38

Dynamic modulation by Trim32 as a novel mechanism for regulating a voltage-gated potassium channel in the brain.

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Despite the strong abundance of Trim32 in the brain, its purpose in the nervous system is poorly understood. Here, we provide evidence that neurological Trim32 modulates Kv1.2, an ion channel key to establishing and regulating neuronal excitability. Specifically, we found that Trim32 interacts with Kv1.2 in the cerebellum where it localizes to distinct regions of high Kv1.2 density. We identified channel ubiquitylation sites within the cerebellum and further demonstrated that Trim32 can ubiquitylate Kv1.2 in vitro, implying Trim32 is the source of modified channel in the brain. We found that overexpression of Trim32 in cultured cells caused channel ubiquitylation which, intriguingly, leads to increased surface expression. Additionally, under separate conditions, Trim32 can decrease surface Kv1.2 independent of channel ubiquitylation, suggesting that Trim32 is a multifaceted regulator of the channel. Finally, we found both mechanisms of Trim32 regulation involve phosphorylation crosstalk within the channel and altogether provide a novel approach to regulation of Kv channels and excitability in the brain.

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Regulation of Cerebellar Kv1.2 by PKM-ζ and its Implication for Learning and Memory

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PKC-ζ and its N-terminal truncated form PKM-ζ, have long been implicated in the regulation of hippocampal AMPA receptors in a cellular correlate of learning, long term potentiation (LTP) (Ling et al., 2002; Yao et al., 2008). Yet little is known about the function of PKM-ζ in the cerebellum. Both PKC-ζ and PKM-ζ are highly expressed in the cerebellum (Oster et al., 2004) and our lab has shown that endocytosis of Kv1.2 in Basket Cells and Purkinje Cells can enhance the acquisition of eye-blink conditioning (EBC) in rats, a form of cerebellar learning (Williams et al., 2012). Having been implicated in the regulation of Kv1.2's β subunit, we hypothesized that PKC-ζ and PKM-ζ may have regulatory effects on Kv1.2, and that this may have implications for cerebellar function. We have shown for the first time that both PKC-ζ and PKM-ζ can alter Kv1.2 surface expression in HEK 293 cells. Furthermore we have shown that inhibition of PKM-ζ/PKC-ζwith ZIP (Zeta-inhibitory peptide) can significantly disrupt eye-blink conditioning in rats.

Poster 40

Intravesical transient receptor potential vanilloid family member 4 (TRPV4) blockade reduces voiding frequency in mice with chronic urothelial overexpression of NGF (NGF-OE).

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Urothelial cells and dorsal root ganglion (DRG) neurons express TRPV4, and roles in normal micturition reflexes as well as micturition dysfunction have been suggested. TRP channel expression and function is dependent upon target tissue expression of growth factors. We have previously demonstrated that TRPV4 transcript and protein expression was increased in the urothelium + suburothelium and suburothelial nerve plexus of the urinary bladder and in small- and medium-sized lumbosacral DRG cells from NGF-OE mice compared to littermate WT mice. NGF-OE mice exhibit increases in NGF transcript and protein in the urothelium + suburothelium and lumbosacral DRG and exhibit increased voiding frequency and non-voiding contractions (NVCs) compared to littermate WT mice. The functional role of TRPV4 in bladder function was evaluated using continuous, open outlet intravesical infusion of saline in conjunction with administration of a TRPV4 antagonist, HC067047 (1 μM) or vehicle (0.1% DMSO in saline) in NGF-OE and WT mice. Bladder capacity, void volume, and intercontraction intervals significantly ($p \leq 0.01$) increased following administration of HC067047 in NGF-OE mice. No changes in bladder function were observed in WT mice with HC067047 or vehicle administration or with administration of vehicle in NGF-OE mice. These results demonstrate that TRPV4 blockade reduces voiding frequency and NVCs in NGF-OE mice consistent with the role of TRPV4 as a target for bladder function disorders.